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【Name of Document】 Claims 1

【Name of Document】 Specification 1

【Name of Document】 Drawings 1

【Name of Document】 Abstract 1

【General Power of Attorney Number】 0216136

[Document Name] Claims

[Claim 1]

An antibody comprising a single-chain polypeptide having binding activity against TPO receptor (Mpl), wherein said antibody comprises two heavy chain variable regions and two light chain variable regions.

[Claim 2]

The antibody of claim 1, wherein the two heavy chain variable regions and the two light chain variable regions are arranged in the order of heavy chain variable region, light chain variable region, heavy chain variable region, and light chain variable region from the N terminus of the single-chain polypeptide.

[Claim 3]

The antibody of claim 1 or 2, wherein the two heavy chain variable regions and the two light chain variable regions are linked by linkers.

[Claim 4]

The antibody of claim 3, wherein the linkers comprise 15 amino acids.

[Claim 5]

A chimeric antibody that binds to Mpl.

[Claim 6]

The antibody of claim 5, which is a humanized antibody.

[Claim 7]

The antibody of claim 5 or 6, which is a minibody.

[Claim 8]

An antibody that binds to soluble Mpl.

[Claim 9]

An antibody that binds to human Mpl and monkey Mpl.

[Claim 10]

An antibody having agonistic activity against human Mpl and monkey Mpl.

[Claim 11]

An antibody whose binding activity to soluble Mpl is KD = 10^{-6} M or lower.

[Claim 12]

An antibody whose binding activity to soluble Mpl is KD = 10^{-7} M or lower.

[Claim 13]

An antibody whose binding activity to soluble Mpl is KD = 10^{-8} M or lower.

[Claim 14]

An antibody whose TPO agonistic activity is EC50 = 100 nM or lower.

[Claim 15]

An antibody whose TPO agonistic activity is EC50 = 30 nM or lower.

[Claim 16]

An antibody whose TPO agonistic activity is EC50 = 10 nM or lower.

[Claim 17]

An antibody which comprises a heavy chain variable region, wherein said heavy chain variable regions comprises CDR1, CDR2 and CDR3 consisting of an amino acid sequence of any one of:

[1] SEQ ID NOs: 3, 4, and 5
[2] SEQ ID NOs: 6, 7, and 8
[3] SEQ ID NOs: 9, 10, and 11
[4] SEQ ID NOs: 15, 16, and 17
[5] SEQ ID NOs: 18, 19, and 20
[6] SEQ ID NOs: 21, 22, and 23
[7] SEQ ID NOs: 24, 25, and 26
[8] SEQ ID NOs: 27, 28, and 29
[9] SEQ ID NOs: 30, 31, and 32
[10] SEQ ID NOs: 33, 34, and 35
[11] SEQ ID NOs: 36, 37, and 38
[12] SEQ ID NOs: 39, 40, and 41
[13] SEQ ID NOs: 42, 43, and 44
[14] SEQ ID NOs: 48, 49, and 50
[15] SEQ ID NOs: 51, 52, and 53
[16] SEQ ID NOs: 54, 55, and 56
[17] SEQ ID NOs: 57, 58, and 59.

[Claim 18]

An antibody which comprises a light chain variable region, wherein said light chain variable region comprises CDR1, CDR2 and CDR3 consisting of an amino acid sequence of any one of:

[1] SEQ ID NOs: 60, 61, and 62
[2] SEQ ID NOs: 63, 64, and 65
[3] SEQ ID NOs: 78, 79, and 80
[4] SEQ ID NOs: 84, 85, and 86
[5] SEQ ID NOs: 93, 94, and 95
[6] SEQ ID NOs: 96, 97, and 98
[7] SEQ ID NOs: 102, 103, and 104
[8] SEQ ID NOs: 108, 109, and 110

[9] SEQ ID NOs: 111, 112, and 113

[10] SEQ ID NOs: 114, 115, and 116.

[Claim 19]

An antibody that comprises a heavy chain variable region and a light chain variable region of any one of:

[1] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 3, 4, and 5, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 60, 61, and 62;

[2] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 6, 7, and 8, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[3] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 9, 10, and 11, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[4] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 15, 16, and 17, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[5] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 18, 19, and 20, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[6] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 21, 22, and 23, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 78, 79, and 80;

[7] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 24, 25, and 26, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[8] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 27, 28, and 29, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting

of SEQ ID NOs: 84, 85, and 86;

[9] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 30, 31, and 32, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[10] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 33, 34, and 35, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[11] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 36, 37, and 38, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 93, 94, and 95;

[12] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 39, 40, and 41, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 96, 97, and 98;

[13] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 42, 43, and 44, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 78, 79, and 80;

[14] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 45, 46, and 47, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 102, 103, and 104;

[15] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 48, 49, and 50, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[16] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 51, 52, and 53, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 108, 109, and 110,

[17] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 54, 55, and 56, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting

of SEQ ID NOs: 111, 112, and 113;

[18] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 57, 58, and 59, and a light chain variable region that comprises CDR1, CDR2, and CDR3 each comprising the amino acid sequences consisting of SEQ ID NOs: 114, 115, and 116.

[Claim 20]

An antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 118.

[Claim 21]

An antibody that comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 120.

[Claim 22]

An antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 118 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 120.

[Claim 23]

An antibody comprising the amino acid sequence of SEQ ID NO: 122 or 264.

[Claim 24]

An antibody that comprises a heavy chain variable region, wherein said heavy chain variable region comprises FR1, FR2, FR3, and FR4 consisting of amino acid sequences of any one of:

[1] SEQ ID NOs: 230, 232, 234, and 236

[2] SEQ ID NOs: 265, 267, 269, and 271

[3] SEQ ID NOs: 279, 281, 283, and 285

[4] SEQ ID NOs: 298, 299, 300, and 301

[5] SEQ ID NOs: 298, 299, 306, and 301.

[Claim 25]

An antibody comprising a light chain variable region, wherein said light chain variable region comprises FR1, FR2, FR3, and FR4 consisting of amino acid sequences of any one of:

[1] SEQ ID NOs: 239, 241, 243, and 245

[2] SEQ ID NOs: 272, 274, 276, and 278

[3] SEQ ID NOs: 302, 303, 304, and 305

[4] SEQ ID NOs: 302, 307, 308, and 305.

[Claim 26]

An antibody that comprises a heavy chain variable region and a light chain variable region of any one of:

[1] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 230, 232, 234, and 236, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 239, 241, 243, and 245;

[2] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 265, 267, 269, and 271, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 272, 274, 276, and 278;

[3] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 279, 281, 283, and 285, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 272, 274, 276, and 278;

[4] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 298, 299, 300, and 301, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 302, 303, 304, and 305;

[5] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 298, 299, 306, and 301, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 302, 307, 308, and 305.

[Claim 27]

An antibody that comprises a heavy chain variable region, wherein said heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 229, 256, 262, 289, or 295.

[Claim 28]

An antibody that comprises a light chain variable region, wherein said light chain variable region comprises the amino acid sequence of SEQ ID NO: 238, 258, 291, or 297.

[Claim 29]

An antibody that comprises a heavy chain variable region and a light chain variable region of any one of:

[1] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 229, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 238;

[2] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 256, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 258;

[3] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 262, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 258;

[4] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:

289, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 291;

[5] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 295, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 297.

[Claim 30]

An antibody that comprises the amino acid sequence of SEQ ID NO: 2, 254, 260, 287, or 293.

[Claim 31]

An antibody having an activity equivalent to that of an antibody of any one of claims 17 to 30, wherein said antibody comprises the amino acid sequence set forth in any one of claims 17 to 30, in which one or more amino acids have been substituted, deleted, added and/or inserted.

[Claim 32]

An antibody that recognizes an epitope recognized by an antibody of any one of claims 17 to 31.

[Claim 33]

An antibody that recognizes the region of amino acids 26 to 274 of human Mpl.

[Claim 34]

An antibody of any one of claims 1 to 33, which has TPO agonistic activity.

[Claim 35]

A polynucleotide encoding an antibody of any one of claims 1 to 34.

[Claim 36]

A polynucleotide hybridizing to the polynucleotide of claim 35 under stringent conditions, wherein said polynucleotide encodes an antibody having activity equivalent to that of an antibody of any one of claims 1 to 34.

[Claim 37]

A vector comprising the polynucleotide of claim 35 or 36.

[Claim 38]

A host cell that carries the polynucleotide of claim 35 or 36, or the vector of claim 37.

[Claim 39]

A pharmaceutical composition comprising an antibody of any one of claims 1 to 34.

[Document Name] Specification

[Title of the Invention] ANTI-MPL ANTIBODIES

[Technical Field]

[0001]

The present invention relates to anti-Mpl antibodies.

[Background of the Invention]

[0002]

Thrombopoietin (TPO) is a factor that enhances the differentiation and maturation of megakaryocytes (platelet precursor cells) from hemopoietic stem cells into platelets. TPO also functions as a cytokine with an important role in the regulation of platelet number. TPO is converted into its active form through the cleavage of a TPO precursor comprising 353 amino acids.

Mpl is a TPO receptor, and human Mpl molecules are known to exist in two forms comprising 572 and 635 amino acids. The human Mpl gene sequence has already been analyzed (see Non-patent Document 1 and GenBank accession No. NM_005373).

Most cytokine receptors dimerize upon ligand binding, and transduce signals into cells. It has been reported that TPO similarly binds to its own specific receptor MPL, which leads to dimerization of the receptor, thereby transducing signals into cells and exerting physiological effects (see Non-patent Document 2).

[0003]

Antibodies exhibiting agonistic activity have been reported among those antibodies that bind to receptors having the above features.

For example, an antibody against the erythropoietin (EPO) receptor has been reported to substitute for erythropoietin function. The monovalent form (Fab) of the antibody is capable of binding to the EPO receptor but is unable to transduce signals. Thus, dimerization of the erythropoietin receptor *via* bivalent binding is assumed to be essential for signal transduction (see Non-patent Document 3).

[0004]

Antibodies that bind to Mpl and exhibit TPO agonistic activity have also been reported (see Non-patent Documents 4 and 5). This suggests that receptor dimerization is induced upon binding of a bivalent antibody with regards to MPL as well.

Meanwhile, a single-chain antibody (scFv) has been reported to exhibit TPO agonistic activity (see Patent Document 1). However, it has been revealed that, the underlying mechanism of scFv exhibiting TPO agonistic activity is that a part of scFv dimerizes (diabody) and this diabody becomes the actual active unit (see Patent Documents 2 to 4).

[0005]

[Patent Document 1] US Patent No. 6342220
[Patent Document 2] WO 01/79494
[Patent Document 3] WO 02/33072
[Patent Document 4] WO 02/33073
[Non-patent Document 1] Palacios *et al.*, 1985, Cell, 41, 727-734
[Non-patent Document 2] Souyri *et al.*, 1990, Cell, Vol.63, 1137-1147
[Non-patent Document 3]Elliott, S. *et al.*, 1996, J. Biol. Chem., 271(40), 24691-24697
[Non-patent Document 4] Abe *et al.*, 1998, Immunol. Lett., 61, 73-78
[Non-patent Document 5] Bijia Deng *et al.*, 1998, Blood, 92, 1981-1988

[Disclosure of the Invention]

[Problems to be Solved by the Invention]

[0006]

The present invention was achieved in view of the above circumstances. An objective of the present invention is to provide novel anti-Mpl antibodies having TPO agonistic activity.

[Means for Solving the Problems]

[0007]

The present inventors performed exhaustive research to solve the above objective. The present inventors prepared and purified anti-human Mpl antibody VB22B, and established a single-chain antibody expression system using genetic engineering techniques. Specifically, the variable region of anti-human Mpl antibody was first cloned, and a diabody expression vector pCXND3-VB22B db for the anti-human Mpl antibody was prepared. This pCXND3-VB22B db vector was then used to generate an expression vector pCXND3-VB22B sc$(Fv)_2$ for anti-human Mpl antibody sc$(Fv)_2$. Anti-human Mpl sc$(Fv)_2$ was expressed in CHO-DG44 cells using the expression vector pCXND3-VB22B sc$(Fv)_2$, and then purified from the culture supernatant. In control experiments, VB22B diabody was transiently expressed in COS7 cells using the above pCXND3-VB22B db vector, and then purified from the culture supernatant.

[0008]

In addition, VB22B diabody and VB22B sc$(Fv)_2$ were evaluated for their TPO-like agonistic activities. The results showed that VB22B diabody and VB22B sc$(Fv)_2$ exhibit higher agonistic activities compared to VB22B IgG, and thus activities equivalent to or higher than that of the natural ligand, human TPO.

Furthermore, the present inventors succeeded in preparing five types of humanized VB22B sc$(Fv)_2$. The TPO-like agonistic activity was also proven to be unaltered by humanization.

[0009]

More specifically, the present invention provides the following (1) to (39):

(1) an antibody comprising a single-chain polypeptide having binding activity against TPO receptor (Mpl), wherein said antibody comprises two heavy chain variable regions and two light chain variable regions;

(2) the antibody of (1), wherein the two heavy chain variable regions and the two light chain variable regions are arranged in the order of heavy chain variable region, light chain variable region, heavy chain variable region, and light chain variable region from the N terminus of the single-chain polypeptide;

(3) the antibody of (1) or (2), wherein the two heavy chain variable regions and the two light chain variable regions are linked by linkers;

(4) the antibody of (3), wherein the linkers comprise 15 amino acids;

(5) a chimeric antibody that binds to Mpl;

(6) the antibody of (5), which is a humanized antibody;

(7) the antibody of (5) or (6), which is a minibody;

(8) an antibody that binds to soluble Mpl;

(9) an antibody that binds to human Mpl and monkey Mpl;

(10) an antibody having agonistic activity against human Mpl and monkey Mpl;

(11) an antibody whose binding activity to soluble Mpl is KD = 10^{-6} M or lower;

(12) an antibody whose binding activity to soluble Mpl is KD = 10^{-7} M or lower;

(13) an antibody whose binding activity to soluble Mpl is KD = 10^{-8} M or lower;

(14) an antibody whose TPO agonistic activity is EC50 = 100 nM or lower;

(15) an antibody whose TPO agonistic activity is EC50 = 30 nM or lower;

(16) an antibody whose TPO agonistic activity is EC50 = 10 nM or lower;

(17) an antibody which comprises a heavy chain variable region, wherein said heavy chain variable regions comprises CDR1, CDR2 and CDR3 consisting of an amino acid sequence of any one of:

[1] SEQ ID NOs: 3, 4, and 5

[2] SEQ ID NOs: 6, 7, and 8

[3] SEQ ID NOs: 9, 10, and 11

[4] SEQ ID NOs: 15, 16, and 17

[5] SEQ ID NOs: 18, 19, and 20

[6] SEQ ID NOs: 21, 22, and 23

[7] SEQ ID NOs: 24, 25, and 26

[8] SEQ ID NOs: 27, 28, and 29

[9] SEQ ID NOs: 30, 31, and 32

[10] SEQ ID NOs: 33, 34, and 35

[11] SEQ ID NOs: 36, 37, and 38

[12] SEQ ID NOs: 39, 40, and 41

[13] SEQ ID NOs: 42, 43, and 44

[14] SEQ ID NOs: 48, 49, and 50

[15] SEQ ID NOs: 51, 52, and 53

[16] SEQ ID NOs: 54, 55, and 56

[17] SEQ ID NOs: 57, 58, and 59;

(18) an antibody which comprises a light chain variable region, wherein said light chain variable region comprises CDR1, CDR2 and CDR3 consisting of an amino acid sequence of any one of:

[1] SEQ ID NOs: 60, 61, and 62

[2] SEQ ID NOs: 63, 64, and 65

[3] SEQ ID NOs: 78, 79, and 80

[4] SEQ ID NOs: 84, 85, and 86

[5] SEQ ID NOs: 93, 94, and 95

[6] SEQ ID NOs: 96, 97, and 98

[7] SEQ ID NOs: 102, 103, and 104

[8] SEQ ID NOs: 108, 109, and 110

[9] SEQ ID NOs: 111, 112, and 113

[10] SEQ ID NOs: 114, 115, and 116;

(19) an antibody that comprises a heavy chain variable region and a light chain variable region of any one of:

[1] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 3, 4, and 5, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 60, 61, and 62;

[2] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 6, 7, and 8, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[3] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 9, 10, and 11, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[4] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 15, 16, and 17, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting

of SEQ ID NOs: 63, 64, and 65;

[5] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 18, 19, and 20, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[6] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 21, 22, and 23, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 78, 79, and 80;

[7] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 24, 25, and 26, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[8] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 27, 28, and 29, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 84, 85, and 86;

[9] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 30, 31, and 32, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[10] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 33, 34, and 35, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[11] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 36, 37, and 38, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 93, 94, and 95;

[12] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 39, 40, and 41, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 96, 97, and 98;

[13] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 42, 43, and 44, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting

of SEQ ID NOs: 78, 79, and 80;

[14] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 45, 46, and 47, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 102, 103, and 104;

[15] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 48, 49, and 50, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 63, 64, and 65;

[16] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 51, 52, and 53, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 108, 109, and 110;

[17] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 54, 55, and 56, and a light chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 111, 112, and 113;

[18] a heavy chain variable region that comprises CDR1, CDR2, and CDR3 comprising the amino acid sequences consisting of SEQ ID NOs: 57, 58, and 59, and a light chain variable region that comprises CDR1, CDR2, and CDR3 each comprising the amino acid sequences consisting of SEQ ID NOs: 114, 115, and 116;

(20) an antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 118;

(21) an antibody that comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 120;

(22) an antibody that comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 118 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 120;

(23) an antibody comprising the amino acid sequence of SEQ ID NO: 122 or 264;

(24) an antibody that comprises a heavy chain variable region, wherein said heavy chain variable region comprises FR1, FR2, FR3, and FR4 consisting of amino acid sequences of any one of:

[1] SEQ ID NOs: 230, 232, 234, and 236

[2] SEQ ID NOs: 265, 267, 269, and 271

[3] SEQ ID NOs: 279, 281, 283, and 285

[4] SEQ ID NOs: 298, 299, 300, and 301

[5] SEQ ID NOs: 298, 299, 306, and 301.

(25) an antibody comprising a light chain variable region, wherein said light chain variable region comprises FR1, FR2, FR3, and FR4 consisting of amino acid sequences of any one of:

[1] SEQ ID NOs: 239, 241, 243, and 245

[2] SEQ ID NOs: 272, 274, 276, and 278

[3] SEQ ID NOs: 302, 303, 304, and 305

[4] SEQ ID NOs: 302, 307, 308, and 305;

(26) an antibody that comprises a heavy chain variable region and a light chain variable region of any one of:

[1] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 230, 232, 234, and 236, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 239, 241, 243, and 245;

[2] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 265, 267, 269, and 271, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 272, 274, 276, and 278;

[3] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 279, 281, 283, and 285, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 272, 274, 276, and 278;

[4] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 298, 299, 300, and 301, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 302, 303, 304, and 305;

[5] a heavy chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 298, 299, 306, and 301, and a light chain variable region which comprises FR1, FR2, FR3, and FR4 having the amino acid sequences consisting of SEQ ID NOs: 302, 307, 308, and 305;

(27) an antibody that comprises a heavy chain variable region, wherein said heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 229, 256, 262, 289, or 295;

(28) an antibody that comprises a light chain variable region, wherein said light chain variable region comprises the amino acid sequence of SEQ ID NO: 238, 258, 291, or 297;

(29) an antibody that comprises a heavy chain variable region and a light chain variable region of any one of:

[1] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 229, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 238;

[2] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 256, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 258;

[3] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 262, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 258;

[4] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 289, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 291;

[5] a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 295, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 297;

(30) an antibody that comprises the amino acid sequence of SEQ ID NO: 2, 254, 260, 287, or 293;

(31) an antibody having an activity equivalent to that of an antibody of any one of (17) to (30), wherein said antibody comprises the amino acid sequence set forth in any one of (17) to (30), in which one or more amino acids have been substituted, deleted, added and/or inserted;

(32) an antibody that recognizes an epitope recognized by an antibody of any one of (17) to (31);

(33) an antibody that recognizes the region of amino acids 26 to 274 of human Mpl;

(34) an antibody of any one of (1) to (33), which has TPO agonistic activity;

(35) a polynucleotide encoding an antibody of any one of (1) to (34);

(36) a polynucleotide hybridizing to the polynucleotide of (35) under stringent conditions, wherein said polynucleotide encodes an antibody having activity equivalent to that of an antibody of any one of claims 1 to 34;

(37) a vector comprising the polynucleotide of (35) or (36);

(38) a host cell that carries the polynucleotide of (35) or (36), or the vector of (37); and

(39) a pharmaceutical composition comprising an antibody of any one of (1) to (34).

[Effects of the Invention]

[0010]

Recombinant human TPOs have been tested as therapeutic agents for chemotherapy-induced thrombocytopenia in various clinical trials. In these trials, a major problem that was reported was the production of anti-TPO antibody in TPO-treated patients (Junzhi Li, *et al.*, Blood (2001) 98: 3241-324; Saroj Vandhan-Raj, *et al.*, Ann. Intern. Med. (2000) 132: 364-368). Specifically, production of neutralizing antibodies that inhibit endogenous TPO activity, and the resulting onset of thrombocytopenia, were reported. Administration of agonistic minibodies (low molecular weight antibodies) against anti-TPO receptor of the present invention does not induce the production of antibodies against

endogenous TPO. Furthermore, since antibody miniaturization results in increased specific activity and short half-life in blood, the effective concentration in blood can be easily regulated, presenting a further clinical advantage. Accordingly, the present antibodies are expected to be therapeutic agents for thrombocytopenia which are more effective than naturally-occurring TPO or its agonistic antibodies. Furthermore, since minibodies are not glycosylated, expression systems for expressing the recombinant proteins are not limited, and minibodies can be produced in any expression system such as cell lines from mammals, yeast, insect cells, and *E. coli.* Since binding specificity against mutant TPO receptor is different from that of TPO, minibodies are expected to bind specific mutants and show agonistic activity against mutated TPO receptor detected in the CAMT patients, who genetically have mutated TPO receptor and develop thrombocytopenia.

[Best Mode for Carrying Out the Invention]

[0011]

The present invention provides antibodies that bind to the TPO receptor (Mpl).

The antibodies of the present invention comprise all types of antibodies, including antibodies with modified amino acid sequences, such as minibodies, humanized antibodies, and chimeric antibodies; antibodies that have been modified by binding with other molecules (for example, polymers such as polyethylene glycol); and antibodies whose sugar chains have been modified.

[0012]

Mpl of the present invention may be a mutant receptor. A mutant receptor of the present invention is usually a receptor that exists at a frequency lower than 50%, preferably lower than 20%, more preferably lower than 10%, and even more preferably lower than 1%. The frequency is generally calculated using randomly selected subjects. However, the frequency may vary depending on the country, area, sex, and such. Therefore, the frequency may also be calculated, for example, within a defined country or area, such as Japan, the United States, and Europe, or calculated for one sex. When there are two or more mutations in a receptor, the frequency may be calculated for multiple mutation sites or for any one of the mutation sites. Mutant receptors are preferably evaluated by a frequency as described above. However, mutant receptors can also be evaluated, for example, by their signal transducing ability and such. Specifically, for example, when two different receptors are present, the one with stronger transducing signals upon natural ligand-binding maybe be used as a non-mutant type receptor, and the one with weaker transducing signals as a mutant receptor.

[0013]

In one embodiment, the mutant receptors of the present invention comprise receptors that are associated with disease onset. The phrase "mutant receptors associated with disease

onset" means that the loss of reactivity to a natural ligand becomes part of the reason that triggers disease onset. In the present invention, the mutant receptor may be a contributing factor, but not necessarily the sole factor triggering disease onset. Many reports have been previously published that describe the association of mutant receptors with disease onset. In addition to those that have been reported, associations of mutant receptors and disease onset can also be identified by statistical analysis methods (for example, correlation analyses). Correlation analyses, also called "case control studies", are well known to those skilled in the art (for example, Nishimura, Y., 1991, "Statistical analysis of polymorphisms", Saishin Igaku, 46:909-923; Oka, A. *et al.*, Hum. Mol. Genetics (1990) 8: 2165-2170; Ota, M. *et al.*, Am. J. Hum. Genet. (1999) 64: 1406-1410; Ozawa, A. *et al.*, Tissue Antigens (1999) 53: 263-268). For example, the correlation between a mutant receptor and a disease can be studied by computing the frequency of the mutant receptor in patients and healthy subjects, and testing whether the patient population has a higher mutant receptor frequency. Typically, differences in frequency are evaluated using the χ-test. χ is obtained by the equation $\chi^2 = \Sigma$ (observed value - expected value)^2/expected value. A p value is obtained from the χ^2 value determined. Based on this p value, it can be determined whether there is a correlation between the mutant receptor and the disease. For example, when $p<0.05$, the mutant receptor is considered to correlate with the disease. Mutant thrombopoietin (TPO) receptors have already been reported (Matthias Ballmaier *et al.*, BLOOD (2001) 97 (1): 139; and others).

[0014]

It is preferable that the antibodies of the present invention have agonistic activity against Mpl.

In a preferred embodiment, the antibodies of the present invention comprise, for example, minibodies.

The minibodies comprise antibody fragments lacking portions of the whole antibody (for example, whole IgG). The minibodies are not particularly limited as long as they have binding activity to their antigens. The minibodies of the present invention have markedly higher activities compared to their corresponding whole antibodies. There are no particular limitations on the antibody fragments of the present invention as long as they are portions of the whole antibody, and preferably contain heavy chain variable regions (VH) and/or light chain variable regions (VL). The amino acid sequences of VH or VL may contain substitutions, deletions, additions and/or insertions. Furthermore, the antibody fragment may also lack portions of VH or/and VL, as long as it has binding ability to its antigen. In addition, the variable regions may be chimerized or humanized. Such antibody fragments include, for example, Fab, Fab', F(ab')$_2$, and Fv. An example of a minibody includes Fab, Fab', F(ab')$_2$, Fv, scFv (single-chain Fv), diabody, and sc(Fv)$_2$ (single-chain (Fv)$_2$).

[0015]

Herein, an "Fv" fragment is the smallest antibody fragment and contains a complete antigen recognition site and a binding site. The "Fv" fragment is a dimer (VH-VL dimer) in which a single VH and a single VL are strongly linked by a non-covalent bond. The three complementarity-determining regions (CDRs) of each of the variable regions interact with each other to form an antigen-binding site on the surface of the VH-VL dimer. Six CDRs confer the antigen-binding site of an antibody. However, a single variable region (or a half of Fv containing only three CDRs specific to an antigen) alone is also capable of recognizing and binding an antigen although its affinity is lower than the affinity of the entire binding site.

[0016]

scFv contains the VH and VL regions of an antibody, and these regions exist on a single polypeptide chain. Generally, an Fv polypeptide further contains a polypeptide linker between VH and VL, and therefore an scFv can form a structure required for antigen binding. See, Pluckthun "The Pharmacology of Monoclonal Antibodies" Vol. 113 (Rosenburg and Moore eds. (Springer Verlag, New York, pp.269-315, 1994) for the review of scFv. In the present invention, linkers are not especially limited as long as they do not inhibit expression of antibody variable regions linked at both ends of the linkers.

[0017]

The term "diabody" refers to a bivalent antibody fragment constructed by gene fusion (Holliger P *et al.*, Proc. Natl. Acad. Sci. USA (1993) 90: 6444-6448; EP 404,097; WO 93/11161 and others). Diabodies are dimers comprising two polypeptide chains, where each polypeptide chain comprises a VL and a VH connected with a linker short enough to prevent interaction of these two domains, for example, a linker of about five residues. The VL and VH encoded on the same polypeptide chain will form a dimer because the linker between them is too short to form a single-chain variable region fragment. As a result, the polypeptide chains form a dimer, and thus the diabody has two antigen binding sites.

[0018]

$sc(Fv)_2$ is a single-chain minibody produced by linking two units of VH and two units of VL with linkers and such (Hudson *et al.*, J Immunol. Methods (1999) 231: 177-189). $sc(Fv)_2$ exhibits a particularly high agonistic activity compared to the whole antibody and other minibodies. $sc(Fv)_2$ can be produced, for example, by linking two scFv molecules.

In a preferable antibody, the two VH units and two VL units are arranged in the order of VH, VL, VH, and VL ([VH]-linker-[VL]-linker-[VH]-linker-[VL]) beginning from the N terminus of a single-chain polypeptide.

[0019]

The order of the two VH units and two VL units is not limited to the above arrangement,

and they may be arranged in any order. Examples of the arrangements are listed below.

[VL]-linker-[VH]-linker-[VH]-linker-[VL]

[VH]-linker-[VL]-linker-[VL]-linker-[VH]

[VH]-linker-[VH]-linker-[VL]-linker-[VL]

[VL]-linker-[VL]-linker-[VH]-linker-[VH]

[VL]-linker-[VH]-linker-[VL]-linker-[VH]

[0020]

The linkers to be used for linking the variable regions of an antibody comprise arbitrary peptide linkers that can be introduced by genetic engineering, synthetic linkers, and linkers disclosed in, for example, Holliger, P. *et al.*, Protein Engineering (1996) 9 (3): 299-305. Peptide linkers are preferred in the present invention. There are no limitations as to the length of the peptide linkers. The length can be selected accordingly by those skilled in the art depending on the purpose, and is typically 1-100 amino acids, preferably 3-50 amino acids, more preferably 5-30 amino acids, and even more preferably 12-18 amino acids (for example, 15 amino acids).

[0021]

For example, such peptide linkers include:

Ser

Gly Ser

Gly Gly Ser

Ser Gly Gly

Gly Gly Gly Ser

Ser Gly Gly Gly

Gly Gly Gly Gly Ser

Ser Gly Gly Gly Gly

Gly Gly Gly Gly Gly Ser

Ser Gly Gly Gly Gly Gly

Gly Gly Gly Gly Gly Gly Ser

Ser Gly Gly Gly Gly Gly Gly

$(Gly\ Gly\ Gly\ Gly\ Ser)_n$

$(Ser\ Gly\ Gly\ Gly\ Gly)_n$

where n is an integer of 1 or larger. The lengths and sequences of peptide linkers can be selected accordingly by those skilled in the art depending on the purpose.

[0022]

In an embodiment of the present invention, a particularly preferable $sc(Fv)_2$ includes, for example, the $sc(Fv)_2$ below.

[VH]-peptide linker (15 amino acids)-[VL]-peptide linker (15 amino acids)-[VH]-peptide linker (15 amino acids)-[VL]

[0023]

Synthetic linkers (chemical crosslinking agents) include crosslinking agents routinely used to crosslink peptides, for example, N-hydroxy succinimide (NHS), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl) suberate (BS^3), dithiobis(succinimidyl propionate) (DSP), dithiobis(sulfosuccinimidyl propionate) (DTSSP), ethylene glycol bis(succinimidyl succinate) (EGS), ethylene glycol bis(sulfosuccinimidyl succinate) (sulfo-EGS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo-DST), bis[2-(succinimidoxycarbonyloxy)ethyl] sulfone (BSOCOES), and bis[2-(sulfosuccinimidoxycarbonyloxy)ethyl] sulfone (sulfo-BSOCOES). These crosslinking agents are commercially available.

[0024]

In general, three linkers are required to link four antibody variable regions together. The linkers to be used may be of the same type or different types. In the present invention, a preferable minibody is a diabody, even more preferably, an $sc(Fv)_2$. Such a minibody can be prepared by treating an antibody with an enzyme, for example, papain or pepsin, to generate antibody fragments, or by constructing DNAs encoding those antibody fragments and introducing them into expression vectors, followed by expression in an appropriate host cell (see, for example, Co, M. S. *et al.*, J. Immunol. (1994) 152: 2968-2976; Better, M. and Horwitz, A. H., Methods Enzymol. (1989) 178: 476-496; Pluckthun, A. and Skerra, A., Methods Enzymol. (1989) 178: 497-515; Lamoyi, E., Methods Enzymol. (1986) 121: 652-663; Rousseaux, J. *et al.*, Methods Enzymol. (1986) 121: 663-669; Bird, R. E. and Walker, B. W., Trends Biotechnol. (1991) 9: 132-137).

[0025]

An antibody having exceedingly high agonistic activity can be prepared by reducing the molecular weight of a full-length antibody, particularly by converting it into an $sc(Fv)_2$.

In a preferred embodiment, the antibodies of the present invention comprise modified antibodies, such as chimeric antibodies and humanized antibodies that bind to Mpl. These modified antibodies can be produced by known methods.

[0026]

Chimeric antibodies are antibodies prepared by combining sequences derived from different animal species, and include for example, antibodies comprising the heavy chain and light chain variable regions of a murine antibody, and the heavy chain and light chain constant regions of a human antibody. Chimeric antibodies can be prepared by known methods. For example, a DNA encoding the V region of an antibody is linked to a DNA encoding the C region of a human antibody, and the construct is inserted into an expression vector and introduced into a

host to produce chimeric antibodies.

[0027]

Humanized antibodies are also referred to as "reshaped human antibodies". Such a humanized antibody is obtained by transferring the complementarity-determining region (CDR) of an antibody derived from a non-human mammal, for example mouse, to the complementarity-determining region of a human antibody, and the general gene recombination procedure for this is also known (see European Patent Application No. 125023 and WO 96/02576).

[0028]

Specifically, a DNA sequence designed to link a murine antibody CDR to the framework region (FR) of a human antibody can be synthesized by PCR, using primers prepared from several oligonucleotides containing overlapping portions of both CDR and FR terminal regions (see methods described in WO 98/13388).

[0029]

The human antibody framework region to be linked by CDR is selected in order to form a favorable antigen-binding site in the complementarity-determining region. Amino acids of the framework region in the antibody variable region may be substituted, as necessary, for the complementarity-determining region of the reshaped human antibody to form a suitable antigen-binding site (Sato, K. *et al.*, Cancer Res. (1993) 53: 851-856).

[0030]

The constant region of a human antibody is used as the constant region of a chimeric antibody or humanized antibody. For example, Cγ1, Cγ2, Cγ3, and Cγ4 can be used as the H chain, and Cκ and Cλ can be used as the L chain. The human antibody constant region may be modified to improve the antibody or the stability of the antibody production.

[0031]

Generally, chimeric antibodies comprise the variable region of an antibody from a non-human mammal and the constant region derived from a human antibody. On the other hand, humanized antibodies comprise the complementarity-determining region of an antibody from a non-human mammal, and the framework region and constant region derived from a human antibody.

In addition, after a chimeric antibody or a humanized antibody is prepared, amino acids in the variable region (for example, FR) and the constant region may be replaced with other amino acids, and such.

[0032]

The origin of the variable regions in chimeric antibodies or that of the CDRs in humanized antibodies is not particularly limited, and may be derived from any type of animal.

For example, sequences of murine antibodies, rat antibodies, rabbit antibodies, camel antibodies may be used.

In general, it is difficult to chimerize or humanize an antibody without losing the agonistic activity of the original antibody. Nevertheless, the present invention succeeded in preparing humanized antibodies having agonistic activity equivalent to that of the original murine antibody.

[0033]

A preferred humanized antibody of the present invention is an antibody comprising a heavy chain variable region that comprises the amino acid sequence of SEQ ID NO: 229 (humanized heavy chain sequence: hVB22B p-z VH), SEQ ID NO: 256 (humanized heavy chain sequence: hVB22B g-e VH), SEQ ID NO: 262 (humanized heavy chain sequence: hVB22B e VH), SEQ ID NO: 289 (humanized heavy chain sequence: hVB22B u2-wz4 VH), or SEQ ID NO: 295 (humanized heavy chain sequence: hVB22B q-wz5 VH); or an antibody comprising a light chain variable region that comprises the amino acid sequence of SEQ ID NO: 238 (humanized light chain hVB22B p-z VL), SEQ ID NO: 258 (humanized light chain hVB22B g-e VL or hVB22B e VL), SEQ ID NO: 291 (humanized light chain hVB22B u2-wz4 VL), or SEQ ID NO: 297 (humanized light chain hVB22B q-wz5 VL). In particular, a preferred antibody is an antibody comprising a heavy chain variable region and a light chain variable region of any one of (1) to (5) indicated below:

(1) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 229, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 238;

(2) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 256, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 258;

(3) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 262, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 258;

(4) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 289, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 291; and

(5) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 295, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 297.

[0034]

Such antibodies include, for example, antibodies comprising the amino acid sequence of SEQ ID NO: 2, 254, 260, 287, or 293 (humanized $sc(Fv)_2$ sequence (hVB22B p-z $sc(Fv)_2$, hVB22B g-e $sc(Fv)_2$, hVB22B e $sc(Fv)_2$, hVB22B u2-wz4, or hVB22B q-wz5).

The nucleotide sequence of hVB22B p-z VH is shown in SEQ ID NO: 228; the nucleotide sequence of hVB22B g-e VH is shown in SEQ ID NO: 255; the nucleotide sequence of hVB22B e VH is shown in SEQ ID NO: 261; the nucleotide sequence of hVB22B u2-wz4 VH

is shown in SEQ ID NO: 288; the nucleotide sequence of hVB22B q-wz5 VH is shown in SEQ ID NO: 294; the nucleotide sequence of hVB22B p-z VL is shown in SEQ ID NO: 237; the nucleotide sequences of hVB22B g-e VL and hVB22B e VL are shown in SEQ ID NO: 257; the nucleotide sequence of hVB22B u2-wz4 VL is shown in SEQ ID NO: 290; and the nucleotide sequence of hVB22B q-wz5 VL is shown in SEQ ID NO: 296.

[0035]

In the amino acid sequence of SEQ ID NO: 229 (humanized heavy chain sequence: hVB22B p-z VH), SEQ ID NO: 256 (humanized heavy chain sequence: hVB22B g-e VH), SEQ ID NO: 262 (humanized heavy chain sequence: hVB22B e VH), SEQ ID NO: 289 (humanized heavy chain sequence: hVB22B u2-wz4 VH), or SEQ ID NO: 295 (humanized heavy chain sequence: hVB22B q-wz5 VH),
amino acids 31-35 correspond to CDR1;
amino acids 50-66 correspond to CDR2;
amino acids 99-107 correspond to CDR3;
amino acids 1-30 correspond to FR1;
amino acids 36-49 correspond to FR2;
amino acids 67-98 correspond to FR3; and
amino acids 108-118 correspond to FR4.

[0036]

In the amino acid sequence of SEQ ID NO: 238 (humanized light chain sequence: hVB22B p-z VL), SEQ ID NO: 258 (humanized light chain sequence: hVB22B g-e VL or hVB22B e VL), SEQ ID NO: 291 (humanized light chain sequence: hVB22B u2-wz4 VL), or SEQ ID NO: 297 (humanized light chain sequence: hVB22B q-wz5 VL),
amino acids 24-39 correspond to CDR1;
amino acids 55-61 correspond to CDR2;
amino acids 94-102 correspond to CDR3;
amino acids 1-23 correspond to FR1;
amino acids 40-54 correspond to FR2;
amino acids 62-93 correspond to FR3; and
amino acids 103-112 correspond to FR4.

[0037]

In the present invention, SEQ ID NOs of the CDRs and FRs in the hVB22B p-z VH sequence are shown below:
hVB22B p-z VH: FR1/SEQ ID NO: 230
hVB22B p-z VH: CDR1/SEQ ID NO: 36
hVB22B p-z VH: FR2/SEQ ID NO: 232

hVB22B p-z VH: CDR2/SEQ ID NO: 37
hVB22B p-z VH: FR3/SEQ ID NO: 234
hVB22B p-z VH: CDR3/SEQ ID NO: 38
hVB22B p-z VH: FR4/SEQ ID NO: 236.

[0038]

In the present invention, SEQ ID NOs of the CDRs and FRs in the hVB22B p-z VL sequence are shown below:

hVB22B p-z VL: FR1/SEQ ID NO: 239
hVB22B p-z VL: CDR1/SEQ ID NO: 93
hVB22B p-z VL: FR2/SEQ ID NO: 241
hVB22B p-z VL: CDR2/SEQ ID NO: 94
hVB22B p-z VL: FR3/SEQ ID NO: 243
hVB22B p-z VL: CDR3/SEQ ID NO: 95
hVB22B p-z VL: FR4/SEQ ID NO: 245.

[0039]

In the present invention, SEQ ID NOs of the CDRs and FRs in the hVB22B g-e VH sequence are shown below:

hVB22B g-e VH: FR1/SEQ ID NO: 265
hVB22B g-e VH: CDR1/SEQ ID NO: 36
hVB22B g-e VH: FR2/SEQ ID NO: 267
hVB22B g-e VH: CDR2/SEQ ID NO: 37
hVB22B g-e VH: FR3/SEQ ID NO: 269
hVB22B g-e VH: CDR3/SEQ ID NO: 38
hVB22B g-e VH: FR4/SEQ ID NO: 271.

[0040]

In the present invention, SEQ ID NOs of the CDRs and FRs in the hVB22B g-e VL sequence are shown below:

hVB22B g-e VL: FR1/SEQ ID NO: 272
hVB22B g-e VL: CDR1/SEQ ID NO: 93
hVB22B g-e VL: FR2/SEQ ID NO: 274
hVB22B g-e VL: CDR2/SEQ ID NO: 94
hVB22B g-e VL: FR3/SEQ ID NO: 276
hVB22B g-e VL: CDR3/SEQ ID NO: 95
hVB22B g-e VL: FR4/SEQ ID NO: 278.

[0041]

In the present invention, SEQ ID NOs of the CDRs and FRs in the hVB22B e VH

sequence are shown below:
hVB22B e VH: FR1/SEQ ID NO: 279
hVB22B e VH: CDR1/SEQ ID NO: 36
hVB22B e VH: FR2/SEQ ID NO: 281
hVB22B e VH: CDR2/SEQ ID NO: 37
hVB22B e VH: FR3/SEQ ID NO: 283
hVB22B e VH: CDR3/SEQ ID NO: 38
hVB22B e VH: FR4/SEQ ID NO: 285.

[0042]

In the present invention, SEQ ID NOs of the CDRs and FRs in the hVB22B e VL sequence are shown below:
hVB22B e VL: FR1/SEQ ID NO: 272
hVB22B e VL: CDR1/SEQ ID NO: 93
hVB22B e VL: FR2/SEQ ID NO: 274
hVB22B e VL: CDR2/SEQ ID NO: 94
hVB22B e VL: FR3/SEQ ID NO: 276
hVB22B e VL: CDR3/SEQ ID NO: 95
hVB22B e VL: FR4/SEQ ID NO: 278.

[0043]

In the present invention, SEQ ID NOs of the CDRs and FRs in the hVB22B u2-wz4 VH sequence are shown below:
hVB22B u2-wz4 VH: FR1/SEQ ID NO: 298
hVB22B u2-wz4 VH: CDR1/SEQ ID NO: 36
hVB22B u2-wz4 VH: FR2/SEQ ID NO: 299
hVB22B u2-wz4 VH: CDR2/SEQ ID NO: 37
hVB22B u2-wz4 VH: FR3/SEQ ID NO: 300
hVB22B u2-wz4 VH: CDR3/SEQ ID NO: 38
hVB22B u2-wz4 VH: FR4/SEQ ID NO: 301.

[0044]

In the present invention, SEQ ID NOs of the CDRs and FRs in the hVB22B u2-wz4 VL sequence are shown below:
hVB22B u2-wz4 VL: FR1/SEQ ID NO: 302
hVB22B u2-wz4 VL: CDR1/SEQ ID NO: 93
hVB22B u2-wz4 VL: FR2/SEQ ID NO: 303
hVB22B u2-wz4 VL: CDR2/SEQ ID NO: 94
hVB22B u2-wz4 VL: FR3/SEQ ID NO: 304

hVB22B u2-wz4 VL: CDR3/SEQ ID NO: 95
hVB22B u2-wz4 VL: FR4/SEQ ID NO: 305.

[0045]

In the present invention, SEQ ID NOs of the CDRs and FRs in the hVB22B q-wz5 VH sequence are shown below:
hVB22B q-wz5 VH: FR1/SEQ ID NO: 298
hVB22B q-wz5 VH: CDR1/SEQ ID NO: 36
hVB22B q-wz5 VH: FR2/SEQ ID NO: 299
hVB22B q-wz5 VH: CDR2/SEQ ID NO: 37
hVB22B q-wz5 VH: FR3/SEQ ID NO: 306
hVB22B q-wz5 VH: CDR3/SEQ ID NO: 38
hVB22B q-wz5 VH: FR4/SEQ ID NO: 301.

[0046]

In the present invention, SEQ ID NOs of the CDRs and FRs in the hVB22B q-wz5 VL sequence are shown below:
hVB22B q-wz5 VL: FR1/SEQ ID NO: 302
hVB22B q-wz5 VL: CDR1/SEQ ID NO: 93
hVB22B q-wz5 VL: FR2/SEQ ID NO: 307
hVB22B q-wz5 VL: CDR2/SEQ ID NO: 94
hVB22B q-wz5 VL: FR3/SEQ ID NO: 308
hVB22B q-wz5 VL: CDR3/SEQ ID NO: 95
hVB22B q-wz5 VL: FR4/SEQ ID NO: 305.

[0047]

SEQ ID NOs of the CDRs and FRs in the hVB22B p-z sequence, hVB22B g-e sequence, hVB22B e sequence, hVB22B u2-wz4 sequence, and hVB22B q-wz5 sequence are shown in Figure 18.

[0048]

In other embodiments, preferred humanized antibodies of the present invention include: humanized antibodies comprising a heavy chain variable region which has FR1, 2, 3, and 4 comprising amino acid sequences of any one of (1) to (5) indicated below:
(1) SEQ ID NOs: 230, 232, 234, and 236 (hVB22B p-z: H chain FR1, 2, 3, and 4),
(2) SEQ ID NOs: 265, 267, 269, and 271 (hVB22B g-e: H chain FR1, 2, 3, and 4),
(3) SEQ ID NOs: 279, 281, 283, and 285 (hVB22B e: H chain FR1, 2, 3, and 4),
(4) SEQ ID NOs: 298, 299, 300, and 301 (hVB22B u2-wz4: H chain FR1, 2, 3, and 4), and
(5) SEQ ID NOs: 298, 299, 306, and 301 (hVB22B q-wz5: H chain FR1, 2, 3, and 4);
humanized antibodies comprising a light chain variable region which has FR1, 2, 3, and 4

comprising amino acid sequences of any one of (1) to (4) listed below:
(1) SEQ ID NOs: 239, 241, 243, and 245 (hVB22B p-z: L chain FR1, 2, 3, and 4),
(2) SEQ ID NOs: 272, 274, 276, and 278 (hVB22B g-e or hVB22B e: L chain FR1, 2, 3, and 4),
(3) SEQ ID NOs: 302, 303, 304, and 305 (hVB22B u2-wz4: L chain FR1, 2, 3, and 4), and
(4) SEQ ID NOs: 302, 307, 308, and 305 (hVB22B q-wz5: L chain FR1, 2, 3, and 4);
humanized antibodies comprising a heavy chain variable region which has CDR1, 2 and 3 comprising amino acid sequences according to the SEQ ID NOs listed below:
SEQ ID NOs: 36, 37, and 38 (hVB22B p-z, hVB22B g-e, hVB22B e, hVB22B u2-wz4, or hVB22B q-wz5: H chain CDR1, 2, and 3); and
humanized antibodies comprising a light chain variable region which has CDR1, 2 and 3 comprising amino acid sequences according to the SEQ ID NOs listed below:
SEQ ID NOs: 93, 94, and 95 (hVB22B p-z hVB22B g-e, hVB22B e, hVB22B u2-wz4, or hVB22B q-wz5: L chain CDR1, 2, and 3).

[0049]

In yet another preferred embodiments, preferred humanized antibodies of the present invention include:
humanized antibodies comprising heavy chain and light chain variable regions of any one of (1) to (5) indicated below.
(1) a heavy chain variable region which comprises FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 230, 232, 234, and 236, respectively, and a light chain variable region which comprises FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 239, 241, 243, and 245, respectively;
(2) a heavy chain variable region which comprises FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 265, 267, 269, and 271, respectively, and a light chain variable region which comprises FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 272, 274, 276, and 278, respectively;
(3) a heavy chain variable region which comprises FR1, 2, 3 and 4 comprising the amino acid sequences of SEQ ID NOs: 279, 281, 283, and 285, respectively, and a light chain variable region which comprises FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 272, 274, 276, and 278, respectively;
(4) a heavy chain variable region which comprises FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 298, 299, 300, and 301, and a light chain variable region which comprises FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 302, 303, 304, and 305, respectively;
(5) a heavy chain variable region which comprises FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 298, 299, 306, and 301, respectively, and a light chain variable

region which comprises FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 302, 307, 308, and 305, respectively; and
humanized antibodies comprising heavy chain and light chain variable regions described below: a heavy chain variable region which comprises CDR1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 36, 37, and 38, respectively, and a light chain variable region which comprises CDR1, 2, and 3 comprising the amino acid sequences of SEQ ID NOs: 93, 94, and 95, respectively.

[0050]

Chimeric antibodies and humanized antibodies exhibit lower antigenicity in the human body, and thus are expected to be useful when administered to humans for therapeutic purposes.

In one embodiment, the preferred antibodies of the present invention include antibodies that bind to soluble Mpl. The term "soluble Mpl" herein refers to Mpl molecules excluding those expressed on the cell membrane. A specific example of a soluble Mpl is an Mpl lacking the entire or a portion of the transmembrane domain. The transmembrane domain of human Mpl corresponds to amino acids 492 to 513 in SEQ ID NO: 123.

[0051]

An antibody that binds to soluble recombinant Mpl can be used in detailed epitope analysis and kinetic analysis of receptor-ligand binding, as well as for assessing the blood concentration and dynamic behavior of the antibody in *in vivo* tests.

[0052]

In one embodiment, the preferred antibodies of the present invention include antibodies having binding activity against both human and monkey Mpl. Antibodies having agonistic activity to both human and monkey Mpl are expected to be highly useful since the dynamic behavior and *in vivo* effects of the antibody, which are generally difficult to determine in human body, can be examined with monkeys.

[0053]

The present invention also provides antibodies having agonistic activity to human Mpl and monkey Mpl. Such antibodies may also have binding activity or agonistic activity against Mpl from animals other than humans and monkeys (for example, mice).

[0054]

In addition, the antibodies of the present invention include antibodies with TPO agonistic activity (agonistic activity against Mpl) of EC50 = 100 nM or lower, preferably EC50 = 30 nM or lower, more preferably EC50 = 10 nM or lower.

[0055]

The agonistic activity can be determined by methods known to those skilled in the art, for example, by the method described below.

The sequences for human Mpl (Palacios *et al.*, Cell (1985) 41: 727-734; GenBank Accession NO. NM_005373), cynomolgus monkey Mpl (the nucleotide sequence and amino acid sequence are shown in SEQ ID NO: 164 and SEQ ID NO: 165, respectively), and mouse Mpl (GenBank Accession NO. NM_010823) are already known.

In addition, the present invention includes antibodies whose binding activities to soluble Mpl are KD = 10^{-6} M or lower, preferably KD = 10^{-7} M or lower, and more preferably KD = 10^{-8} M or lower.

[0056]

In the present invention, whether the binding activity of an antibody to soluble recombinant Mpl is KD = 10^{-6} M or lower can be determined by methods known to those skilled in the art. For example, the activity can be determined using surface plasmon resonance with Biacore. Specifically, soluble MPL-Fc protein is immobilized onto sensor chips. Reaction rate constant can be determined by assessing the interaction between the antibody and the soluble Mpl-Fc protein. The binding activity can be evaluated by ELISA (enzyme-linked immunosorbent assays), EIA (enzyme immunoassays), RIA (radio immunoassays), or fluorescent antibody techniques. For example, in enzyme immunoassays, a sample containing a test antibody, such as purified antibody or culture supernatant of cells producing the test antibody, is added to a plate coated with an antigen to which the test antibody can bind. After incubating the plate with a secondary antibody labeled with an enzyme such as alkaline phosphatase, the plate is washed and an enzyme substrate such as p-nitrophenyl phosphate is added. The antigen-binding activity can then be evaluated by determining the absorbance.

[0057]

There is no specific limitation as to the upper limit of the binding activity; for example, the upper limit may be set within a technically feasible range by those skilled in the art. However, the technically feasible range may expand with the advancement of technology.

[0058]

In an embodiment, the preferred antibodies of the present invention include antibodies recognizing epitopes that are recognized by any one of the antibodies indicated in (I) to (XII) below. The antibody of any one of (I) to (XII) is preferably a minibody.

[0059]

(I)

Antibody comprising a VH that has CDR1, 2, and 3 comprising the amino acid sequences according to SEQ ID NOs in any one of (1) to (17) indicated below (name of each antibody and the H chain CDR contained in the antibody are indicated inside the parentheses):

(1) SEQ ID NOs: 3, 4, and 5 (VA7: H chain CDR1, 2, and 3),

(2) SEQ ID NOs: 6, 7, and 8 (VA130 or VB17B: H chain CDR1, 2, and 3),

(3) SEQ ID NOs: 9, 10, and 11 (VA259: H chain CDR1, 2, and 3),
(4) SEQ ID NOs: 15, 16, and 17 (VB12B: H chain CDR1, 2, and 3),
(5) SEQ ID NOs: 18, 19, and 20 (VB140: H chain CDR1, 2, and 3),
(6) SEQ ID NOs: 21, 22, and 23 (VB33: H chain CDR1, 2, and 3),
(7) SEQ ID NOs: 24, 25, and 26 (VB45B: H chain CDR1, 2, and 3),
(8) SEQ ID NOs: 27, 28, and 29 (VB8B: H chain CDR1, 2, and 3),
(9) SEQ ID NOs: 30, 31, and 32 (VB115: H chain CDR1, 2, and 3),
(10) SEQ ID NOs: 33, 34, and 35 (VB14B: H chain CDR1, 2, and 3),
(11) SEQ ID NOs: 36, 37, and 38 (VB22B, VB4B, hVB22B p-z, hVB22B g-e, hVB22B e, hVB22B u2-wz4, or hVB22B q-wz5: H chain CDR1, 2, and 3),
(12) SEQ ID NOs: 39, 40, and 41 (VB16: H chain CDR1, 2, and 3),
(13) SEQ ID NOs: 42, 43, and 44 (VB157: H chain CDR1, 2, and 3),
(14) SEQ ID NOs: 48, 49, and 50 (VB51: H chain CDR1, 2, and 3),
(15) SEQ ID NOs: 51, 52, and 53 (AB317: H chain CDR1, 2, and 3),
(16) SEQ ID NOs: 54, 55, and 56 (AB324: H chain CDR1, 2, and 3),
(17) SEQ ID NOs: 57, 58, and 59 (TA136: H chain CDR1, 2, and 3).

[0060]

(II)

Antibody comprising a VL which has CDR1, 2, and 3 comprising the amino acid sequences according to SEQ ID NOs in any one of (1) to (10) indicated below (name of each antibody and the L chain CDR in the antibody are indicated inside the parentheses):
(1) SEQ ID NOs: 60, 61, and 62 (VA7: L chain CDR1, 2, and 3),
(2) SEQ ID NOs: 63, 64, and 65 (VA130, VA259, VB17B, VB12B, VB140, VB45B, VB115, VB14B, or VB51: L chain CDR1, 2, and 3),
(3) SEQ ID NOs: 78, 79, and 80 (VB33 or VB157: L chain CDR1, 2, and 3),
(4) SEQ ID NOs: 84, 85, and 86 (VB8B: L chain CDR1, 2, and 3),
(5) SEQ ID NOs: 93, 94, and 95 (VB22B, hVB22B p-z, hVB22B g-e, hVB22B e, hVB22B u2-wz4 or hVB22B q-wz5: L chain CDR1, 2, and 3),
(6) SEQ ID NOs: 96, 97, and 98 (VB16: L chain CDR1, 2, and 3),
(7) SEQ ID NOs: 102, 103, and 104 (VB4B: L chain CDR1, 2, and 3),
(8) SEQ ID NOs: 108, 109, and 110 (AB317: L chain CDR1, 2, and 3),
(9) SEQ ID NOs: 111, 112, and 113 (AB324: L chain CDR1, 2, and 3),
(10) SEQ ID NOs: 114, 115, and 116 (TA136: L chain CDR1, 2, and 3).

[0061]

(III)

Antibody comprising a VH that comprises an amino acid sequence of the SEQ ID NO in

any one of (1) to (24):
(1) SEQ ID NO: 124 (VA7: VH),
(2) SEQ ID NO: 126 (VA130: VH),
(3) SEQ ID NO: 128 (VA259: VH),
(4) SEQ ID NO: 130 (VB17B: VH),
(5) SEQ ID NO: 132 (VB12B: VH),
(6) SEQ ID NO: 134 (VB140: VH),
(7) SEQ ID NO: 136 (VB33: VH),
(8) SEQ ID NO: 138 (VB45B: VH),
(9) SEQ ID NO: 140 (VB8B: VH),
(10) SEQ ID NO: 142 (VB115: VH),
(11) SEQ ID NO: 144 (VB14B: VH),
(12) SEQ ID NO: 118 (VB22B: VH),
(13) SEQ ID NO: 146 (VB16: VH),
(14) SEQ ID NO: 148 (VB157: VH),
(15) SEQ ID NO: 150 (VB4B: VH),
(16) SEQ ID NO: 152 (VB51: VH),
(17) SEQ ID NO: 155 (AB317: VH),
(18) SEQ ID NO: 159 (AB324: VH),
(19) SEQ ID NO: 162 (TA136: VH),
(20) SEQ ID NO: 229 (hVB22B p-z: VH),
(21) SEQ ID NO: 256 (hVB22B g-e: VH),
(22) SEQ ID NO: 262 (hVB22B e: VH),
(23) SEQ ID NO: 289 (hVB22B u2-wz4: VH),
(24) SEQ ID NO: 295 (hVB22B q-wz5: VH).

[0062]

(IV)

Antibody comprising a VL that comprises an amino acid sequence of the SEQ ID NO in any one of (1) to (18):
(1) SEQ ID NO: 125 (VA7: VL),
(2) SEQ ID NO: 127 (VA130, VB17B, VB12B, VB115, or VB14B: VL),
(3) SEQ ID NO: 129 (VA259: VL),
(4) SEQ ID NO: 135 (VB140 or VB45B: VL),
(5) SEQ ID NO: 137 (VB33: VL),
(6) SEQ ID NO: 141 (VB8B: VL),
(7) SEQ ID NO: 120 (VB22B: VL),

(8) SEQ ID NO: 147 (VB16: VL),
(9) SEQ ID NO: 149 (VB157: VL),
(10) SEQ ID NO: 151 (VB4B: VL),
(11) SEQ ID NO: 153 (VB51: VL),
(12) SEQ ID NO: 157 (AB317: VL),
(13) SEQ ID NO: 161 (AB324: VL),
(14) SEQ ID NO: 163 (TA136: VL),
(15) SEQ ID NO: 238 (hVB22B p-z: VL),
(16) SEQ ID NO: 258 (hVB22B g-e: VL or hVB22B e: VL),
(17) SEQ ID NO: 291 (hVB22B u2-wz4: VL),
(18) SEQ ID NO: 297 (hVB22B q-wz5: VL).

[0063]

(V)

Antibody comprising a VH and VL according to any one of (1) to (18):

(1) SEQ ID NOs: 3, 4, and 5 (VA7: H chain CDR1, 2, and 3); SEQ ID NOs: 60, 61, and 62 (VA7: L chain CDR1, 2, and 3),
(2) SEQ ID NOs: 6, 7, and 8 (VA130 or VB17B: H chain CDR1, 2, and 3), SEQ ID NOs: 63, 64, and 65 (VA130 or VB17B: L chain CDR1, 2, and 3),
(3) SEQ ID NOs: 9, 10, and 11 (VA259: H chain CDR1, 2, and 3); SEQ ID NOs: 66, 67, and 68 (VA259: L chain CDR1, 2, and 3),
(4) SEQ ID NOs: 15, 16, and 17 (VB12B: H chain CDR1, 2, and 3); SEQ ID NOs: 72, 73, and 74 (VB12B: L chain CDR1, 2, and 3),
(5) SEQ ID NOs: 18, 19, and 20 (VB140: H chain CDR1, 2, and 3); SEQ ID NOs: 75, 76, and 77 (VB140: L chain CDR1, 2, and 3),
(6) SEQ ID NOs: 21, 22, and 23 (VB33: H chain CDR1, 2, and 3); SEQ ID NOs: 78, 79, and 80 (VB33: L chain CDR1, 2, and 3),
(7) SEQ ID NOs: 24, 25, and 26 (VB45B: H chain CDR1, 2, and 3); SEQ ID NOs: 81, 82, and 83 (VB45B: L chain CDR1, 2, and 3),
(8) SEQ ID NOs: 27, 28, and 29 (VB8B: H chain CDR1, 2, and 3); SEQ ID NOs: 84, 85, and 86 (VB8B: L chain CDR1, 2, and 3),
(9) SEQ ID NOs: 30, 31, and 32 (VB115: H chain CDR1, 2, and 3); SEQ ID NOs: 87, 88, and 89 (VB115: L chain CDR1, 2, and 3),
(10) SEQ ID NOs: 33, 34, and 35 (VB14B: H chain CDR1, 2, and 3); SEQ ID NOs: 90, 91, and 92 (VB14B: L chain CDR1, 2, and 3),
(11) SEQ ID NOs: 36, 37, and 38 (VB22B, hVB22B p-z, hVB22B g-e, hVB22B e, hVB22B u2-wz4, or hVB22B q-wz5: H chain CDR1, 2, and 3); SEQ ID NOs: 93, 94, and 95 (VB22B,

hVB22B p-z, hVB22B g-e, hVB22B e, hVB22B u2-wz4, or hVB22B q-wz5: L chain CDR1, 2, and 3),

(12) SEQ ID NOs: 39, 40, and 41 (VB16: H chain CDR1, 2, and 3); SEQ ID NOs: 96, 97, and 98 (VB16: L chain CDR1, 2, and 3),

(13) SEQ ID NOs: 42, 43, and 44 (VB157: H chain CDR1, 2, and 3); SEQ ID NOs: 99, 100, and 101 (VB157: L chain CDR1, 2, and 3),

(14) SEQ ID NOs: 45, 46, and 47 (VB4B: H chain CDR1, 2, and 3); SEQ ID NOs: 102, 103, and 104 (VB4B: L chain CDR1, 2, and 3),

(15) SEQ ID NOs: 48, 49, and 50 (VB51: H chain CDR1, 2, and 3); SEQ ID NOs: 105, 106, and 107 (VB51: L chain CDR1, 2, and 3),

(16) SEQ ID NOs: 51, 52, and 53 (AB317: H chain CDR1, 2, and 3); SEQ ID NOs: 108, 109, and 110 (AB317: L chain CDR1, 2, and 3),

(17) SEQ ID NOs: 54, 55, and 56 (AB324: H chain CDR1, 2, and 3); SEQ ID NOs: 111, 112, and 113 (AB324: L chain CDR1, 2, and 3),

(18) SEQ ID NOs: 57, 58, and 59 (TA136: H chain CDR1, 2, and 3); SEQ ID NOs: 114, 115, and 116 (TA136: L chain CDR1, 2, and 3).

[0064]

(VI)

Antibody comprising a VH and a VL that comprise the amino acid sequences according to SEQ ID NOs in any one of (1) to (24) indicated below:

(1) SEQ ID NO: 124 (VA7: VH), SEQ ID NO: 125 (VA7: VL),

(2) SEQ ID NO: 126 (VA130: VH), SEQ ID NO: 127 (VA130: VL),

(3) SEQ ID NO: 128 (VA259: VH), SEQ ID NO: 129 (VA259: VL),

(4) SEQ ID NO: 130 (VB17B: VH), SEQ ID NO: 127 (VB17B: VL),

(5) SEQ ID NO: 132 (VB12B: VH), SEQ ID NO: 127 (VB12B: VL),

(6) SEQ ID NO: 134 (VB140: VH), SEQ ID NO: 135 (VB140: VL),

(7) SEQ ID NO: 136 (VB33: VH), SEQ ID NO: 137 (VB33: VL),

(8) SEQ ID NO: 138 (VB45B: VH), SEQ ID NO: 135 (VB45B: VL),

(9) SEQ ID NO: 140 (VB8B: VH), SEQ ID NO: 141 (VB8B: VL),

(10) SEQ ID NO: 142 (VB115: VH), SEQ ID NO: 127 (VB115: VL),

(11) SEQ ID NO: 144 (VB14B: VH), SEQ ID NO: 127 (VB14B: VL),

(12) SEQ ID NO: 118 (VB22B: VH), SEQ ID NO: 120 (VB22B: VL),

(13) SEQ ID NO: 146 (VB16: VH), SEQ ID NO: 147 (VB16: VL),

(14) SEQ ID NO: 148 (VB157: VH), SEQ ID NO: 149 (VB157: VL),

(15) SEQ ID NO: 150 (VB4B: VH), SEQ ID NO: 151 (VB4B: VL),

(16) SEQ ID NO: 152 (VB51: VH), SEQ ID NO: 153 (VB51: VL),

(17) SEQ ID NO: 155 (AB317: VH), SEQ ID NO: 157 (AB317: VL),
(18) SEQ ID NO: 159 (AB324: VH), SEQ ID NO: 161 (AB324: VL),
(19) SEQ ID NO: 162 (TA136: VH), SEQ ID NO: 163 (TA136: VL),
(20) SEQ ID NO: 229 (hVB22B p-z: VH), SEQ ID NO: 238 (hVB22B p-z: VL),
(21) SEQ ID NO: 256 (hVB22B g-e: VH), SEQ ID NO: 258 (hVB22B g-e: VL),
(22) SEQ ID NO: 262 (hVB22B e: VH), SEQ ID NO: 258 (hVB22B e: VL),
(23) SEQ ID NO: 289 (hVB22B u2-wz4: VH), SEQ ID NO: 291 (hVB22B u2-wz4: VL),
(24) SEQ ID NO: 295 (hVB22B q-wz5: VH), SEQ ID NO: 297 (hVB22B q-wz5: VL).

[0065]

(VII)

Antibody comprising the amino acid sequence of SEQ ID NO: 122 (VB22B: scFv).

[0066]

(VIII)

Humanized antibody comprising an amino acid sequence according to any one of SEQ ID NO: 2 (hVB22B p-z: $sc(Fv)_2$), SEQ ID NO: 254 (hVB22B g-e: $sc(Fv)_2$), SEQ ID NO: 260 (hVB22B e: $sc(Fv)_2$), SEQ ID NO: 287 (hVB22B u2-wz4: $sc(Fv)_2$), and SEQ ID NO: 293 (hVB22B q-wz5: $sc(Fv)_2$).

[0067]

(IX)

Antibody comprising a VH which has:
(1) SEQ ID NOs: 230, 232, 234, and 236 (hVB22B p-z: H chain FR1, 2, 3, and 4),
(2) SEQ ID NOs: 265, 267, 269, and 271 (hVB22B g-e: H chain FR1, 2, 3, and 4),
(3) SEQ ID NOs: 279, 281, 283, and 285 (hVB22B e: H chain FR1, 2, 3, and 4),
(4) SEQ ID NOs: 298, 299, 300, and 301 (hVB22B u2-wz4: H chain FR1, 2, 3, and 4),
(5) SEQ ID NOs: 298, 299, 306, and 301 (hVB22B q-wz5: H chain FR1, 2, 3, and 4).

(X)

[0068]

Antibody comprising a VL which has FR1, 2, 3 and 4 comprising amino acid sequences according to SEQ ID NOs in any one of (1) to (4) indicated below:
(1) SEQ ID NOs: 239, 241, 243, and 245 (hVB22B p-z: L chain FR1, 2, 3, and 4),
(2) SEQ ID NOs: 272, 274, 276, and 278 (hVB22B g-e or hVB22B e: L chain FR1, 2, 3, and 4),
(3) SEQ ID NOs: 302, 303, 304, and 305 (hVB22B u2-wz4: L chain FR1, 2, 3, and 4),
(4) SEQ ID NOs: 302, 307, 308, and 305 (hVB22B q-wz5: L chain FR1, 2, 3, and 4).

(XI)

[0069]

Antibody comprising VH and VL according to any one of (1) to (5) indicated below:

(1) VH having FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 230, 232, 234, and 236, respectively, and VL having FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 239, 241, 243, and 245, respectively;
(2) VH having FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 265, 267, 269, and 271, respectively, and VL having FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 272, 274, 276, and 278, respectively;
(3) VH having FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 279, 281, 283, and 285, respectively, and VL having FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 272, 274, 276, and 278, respectively;
(4) VH having FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 298, 299, 300, and 301, respectively, and VL having FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 302, 303, 304, and 305, respectively;
(5) VH having FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 298, 299, 306, and 301, respectively, and VL having FR1, 2, 3, and 4 comprising the amino acid sequences of SEQ ID NOs: 302, 307, 308, and 305, respectively.

[0070]

(XII)

Antibody comprising the amino acid sequence of SEQ ID NO: 264 (VB22B: $sc(Fv)_2$).

[0071]

An antibody comprising an amino acid sequence of any one of (I) to (XII) indicated above, in which one or more amino acids have been substituted, deleted, added, and/or inserted, wherein the antibody has activity equivalent to that of the antibody of any one of (I) to (XII).

Herein, the phrase "functionally equivalent" means that an antibody of interest has a biological or biochemical activity comparable to that of an antibody of the present invention. Such activities include, for example, binding activities and agonistic activities.

[0072]

Methods for preparing polypeptides functionally equivalent to a certain polypeptide are well known to those skilled in the art, and include methods of introducing mutations into polypeptides. For example, those skilled in the art can prepare an antibody functionally equivalent to the antibodies of the present invention by introducing appropriate mutations into the antibody using site-directed mutagenesis (Hashimoto-Gotoh, T. *et al.* Gene (1995) 152: 271-275; Zoller, MJ, and Smith, M. Methods Enzymol. (1983) 100: 468-500; Kramer, W. *et al.*, Nucleic Acids Res. (1984) 12: 9441-9456; Kramer, W. and Fritz HJ, Methods Enzymol. (1987) 154: 350-367; Kunkel, TA, Proc. Natl. Acad. Sci. USA (1985) 82: 488-492; Kunkel, Methods Enzymol. (1988) 85: 2763-2766), or such. Amino acid mutations may occur naturally. Thus, the present invention also comprises antibodies functionally equivalent to the antibodies of the

present invention and comprising the amino acid sequences of these antibodies, in which one or more amino acids is mutated. Generally, the number of amino acids that are mutated is 50 amino acids or less, preferably 30 or less, more preferably 10 or less (for example, five amino acids or less).

[0073]

An amino acid is preferably substituted for a different amino acid(s) that allows the properties of the amino acid side-chain to be conserved. Examples of amino acid side chain properties are: hydrophobic amino acids (A, I, L, M, F, P, W, Y, and V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, and T), amino acids comprising the following side chains: aliphatic side chains (G, A, V, L, I, and P); hydroxyl-containing side chains (S, T, and Y); sulfur-containing side chains (C and M); carboxylic acid- and amide-containing side chains (D, N, E, and Q); basic side chains (R, K, and H); aromatic ring-containing side chains (H, F, Y, and W) (amino acids are represented by one-letter codes in parentheses).

[0074]

A polypeptide comprising a modified amino acid sequence, in which one or more amino acid residues is deleted, added, and/or replaced with other amino acids, is known to retain its original biological activity (Mark, D. F. *et al.*, Proc. Natl. Acad. Sci. USA (1984) 81: 5662-5666; Zoller, M. J. & Smith, M. Nucleic Acids Research (1982) 10: 6487-6500; Wang, A. *et al.*, Science 224, 1431-1433; Dalbadie-McFarland, G. *et al.*, Proc. Natl. Acad. Sci. USA (1982) 79: 6409-6413).

[0075]

Fusion proteins containing antibodies that comprise the amino acid sequence of an antibody of the present invention, in which two or more amino acid residues have been added, are included in the present invention. The fusion protein results from a fusion between one of the above antibodies and a second peptide or protein, and is included in the present invention. The fusion protein can be prepared by ligating a polynucleotide encoding an antibody of the present invention and a polynucleotide encoding a second peptide or polypeptide in frame, inserting this into an expression vector, and expressing the fusion construct in a host. Some techniques known to those skilled in the art are available for this purpose. The partner peptide or polypeptide to be fused with an antibody of the present invention may be a known peptide, for example, FLAG (Hopp, T. P. *et al.*, BioTechnology (1988) 6: 1204-1210), 6x His consisting of six His (histidine) residues, 10x His, influenza hemagglutinin (HA), human c-myc fragment, VSV-GP fragment, p18HIV fragment, T7-tag, HSV-tag, E-tag, SV40 T antigen fragment, lck tag, α-tubulin fragment, B-tag, Protein C fragment. Other partner polypeptides to be fused with the antibodies of the present invention include, for example, GST (glutathione-S-transferase), HA (influenza hemagglutinin), immunoglobulin constant region, β-galactosidase, and MBP

(maltose-binding protein). A polynucleotide encoding one of these commercially available peptides or polypeptides can be fused with a polynucleotide encoding an antibody of the present invention. The fusion polypeptide can be prepared by expressing the fusion construct.

[0076]

As described below, the antibodies of the present invention may differ in amino acid sequence, molecular weight, isoelectric point, presence/absence of sugar chains, and conformation depending on the cell or host producing the antibody, or purification method. However, a resulting antibody is included in the present invention, as long as it is functionally equivalent to an antibody of the present invention. For example, when an antibody of the present invention is expressed in prokaryotic cells, for example *E. coli*, a methionine residue is added to the N terminus of the original antibody amino acid sequence. Such antibodies are included in the present invention.

[0077]

An antibody that recognizes an epitope recognized by the antibody according to any one of (I) to (XII) indicated above is expected to have a high agonistic activity. Such antibodies can be prepared by methods known to those skilled in the art. The antibody can be prepared by, for example, determining the epitope recognized by the antibody according to any one of (I) to (XII) by conventional methods, and using a polypeptide comprising one of the epitope amino acid sequences as an immunogen. Alternatively, the antibody can be prepared by determining the epitopes of conventionally prepared antibodies and selecting an antibody that recognizes the epitope recognized by an antibody of any one of (I) to (XII).

[0078]

In the present invention, a particularly preferred antibody is an antibody that recognizes the epitope recognized by the antibody comprising the amino acid sequence of SEQ ID NO: 2. The antibody comprising the amino acid sequence of SEQ ID NO: 2 is predicted to recognize the region from Glu 26 to Leu 274, preferably the region from Ala 189 to Gly 245, more preferably the region from Gln 213 to Ala 231 of human Mpl. Thus, antibodies recognizing the region of amino acids 26 to 274, or amino acids 189 to 245, or amino acids 213 to 231 of human Mpl are also included in the present invention.

[0079]

Antibodies recognizing regions of amino acids 26 to 274, amino acids 189 to 245, or amino acids 213 to 231 of the human Mpl amino acid sequence (SEQ ID NO: 123) can be obtained by methods known to those skilled in the art. Such antibodies can be prepared by, for example, using a peptide comprising amino acids 26 to 274, amino acids 189 to 245, or amino acids 213 to 231 of the human Mpl amino acid sequence (SEQ ID NO: 123) as an immunogen. Alternatively, such antibodies can be prepared by determining the epitope of a conventionally

prepared antibody and selecting an antibody that recognizes the same epitope recognized by an antibody of the present invention.

[0080]

The present invention provides antibodies described above in (I) to (XII). In an embodiment of the present invention, a preferred antibody is the one shown in (V), a more preferred antibody is the one shown in (VI), and a still more preferred is the one shown in (VIII).

[0081]

The present invention also provides vectors comprising polynucleotides encoding the antibodies of the present invention, or polynucleotides which hybridize under stringent conditions to the polynucleotides of the present invention and encode antibodies having activities equivalent to those of the antibodies of the present invention. The polynucleotides of the present invention are polymers comprising multiple bases or base pairs of deoxyribonucleic acids (DNA) or ribonucleic acids (RNA), and are not particularly limited, as long as they encode the antibodies of the present invention. They may also contain non-natural nucleotides. The polynucleotides of the present invention can be used to express antibodies using genetic engineering techniques. The polynucleotides of this invention can also be used as probes in the screening of antibodies functionally equivalent to the antibodies of the present invention. Specifically, DNAs that hybridize under stringent conditions to a polynucleotide encoding an antibody of the present invention, and encode antibodies having activity equivalent to those of the antibodies of the present invention can be obtained by techniques such as hybridization and gene amplification (for example, PCR), using a polynucleotide of the present invention or a portion thereof as a probe. Such DNAs are also included in the polynucleotides of the present invention. Hybridization techniques are well known to those skilled in the art (Sambrook, J *et al.*, Molecular Cloning 2nd ed., 9.47-9.58, Cold Spring Harbor Lab. press, 1989). Such hybridization conditions include, for example, conditions of low stringency. Examples of conditions of low stringency include post-hybridization washing in 0.1x SSC and 0.1% SDS at 42°C, and preferably in 0.1x SSC and 0.1% SDS at 50°C. More preferable hybridization conditions include those of high stringency. Highly stringent conditions include, for example, washing in 5x SSC and 0.1% SDS at 65°C. In these conditions, the higher the temperature, the higher the expectation of efficiently obtaining polynucleotides with a high homology. However, several factors, such as temperature and salt concentration, can influence hybridization stringency, and those skilled in the art can suitably select these factors to accomplish similar stringencies.

[0082]

Antibodies that are encoded by polynucleotides obtained by the hybridization and gene amplification techniques, and are functionally equivalent to the antibodies of the present

invention generally exhibit high homology to the antibodies of the this invention at the amino acid level. The antibodies of the present invention include antibodies that are functionally equivalent to the antibodies of the present invention, and exhibit high amino acid sequence homology to the antibodies of this invention. The term "high homology" generally means identity at the amino acid level of at least 50% or higher, preferably 75% or higher, more preferably 85% or higher, still more preferably 95% or higher. Polypeptide homology can be determined by the algorithm described in the report: Wilbur, W. J. and Lipman, D. J. Proc. Natl. Acad. Sci. USA (1983) 80: 726-730.

[0083]

When *E. coli* is used as a host, there is no particular limitation as to the type of vector of the present invention, as long as the vector contains an "ori" responsible for its replication in *E. coli* and a marker gene. The "ori" ensures the amplification and mass production of the vector in *E. coli* (for example, JM109, DH5α, HB101, and XL1Blue). The marker gene is used to select the *E. coli* transformants (for example, a drug resistance gene selected by an appropriate drug such as ampicillin, tetracycline, kanamycin, and chloramphenicol). The vectors include, for example, M13 vectors, pUC vectors, pBR322, pBluescript, and pCR-Script. In addition to the above vectors, for example, pGEM-T, pDIRECT, and pT7 can also be used for the subcloning and excision of cDNAs.

[0084]

An expression vector is especially useful for the type of vectors of the present invention. When an expression vector is expressed, for example, in *E. coli*, it should have the above characteristics in order to be amplified in *E. coli*. Additionally, when *E. coli*, such as JM109, DH5α, HB101, or XL1-Blue are used as the host cell, the vector preferably has a promoter, for example, lacZ promoter (Ward *et al.* Nature (1989) 341: 544-546; FASEB J. (1992) 6: 2422-2427), araB promoter (Better *et al.*, Science (1988) 240: 1041-1043), or T7 promoter, that allows efficient expression of the desired gene in *E. coli*. Other examples of the vectors include pGEX-5X-1 (Pharmacia), "QIAexpress system" (QIAGEN), pEGFP, and pET (where BL21, a strain expressing T7 RNA polymerase, is preferably used as the host).

[0085]

Furthermore, the vector may comprise a signal sequence for polypeptide secretion. When producing polypeptides into the periplasm of *E. coli*, the pelB signal sequence (Lei, S. P. *et al.*, J. Bacteriol. (1987) 169: 4379) may be used as a signal sequence for polypeptide secretion. For example, calcium chloride methods or electroporation methods may be used to introduce the vector into a host cell.

[0086]

In addition to *E. coli*, expression vectors derived from mammals (e.g., pCDNA3

(Invitrogen), pEGF-BOS (Nucleic Acids Res. (1990) 18 (17): 5322), pEF, pCDM8), insect cells (e.g., "Bac-to-BAC baculovirus expression system" (GIBCO-BRL), pBacPAK8), plants (e.g., pMH1, pMH2), animal viruses (e.g., pHSV, pMV, pAdexLcw), retroviruses (e.g., pZIPneo), yeasts (e.g., "Pichia Expression Kit" (Invitrogen), pNV11, SP-Q01), and *Bacillus subtilis* (e.g., pPL608, pKTH50) may also be used as a vector of the present invention.

[0087]

In order to express proteins in animal cells such as CHO, COS, and NIH3T3 cells, the vector preferably has a promoter necessary for expression in such cells, for example, an SV40 promoter (Mulligan *et al.*, Nature (1979) 277: 108), MMLV-LTR promoter, EF1α promoter (Mizushima *et al.*, Nucleic Acids Res. (1990) 18: 5322), CMV promoter, etc.). It is even more preferable that the vector also carries a marker gene for selecting transformants (for example, a drug-resistance gene selected by a drug such as neomycin and G418). Examples of vectors with such characteristics include pMAM, pDR2, pBK-RSV, pBK-CMV, pOPRSV, and pOP13, and such.

[0088]

In addition, to stably express a gene and amplify the gene copy number in cells, CHO cells that are defective in the nucleic acid synthesis pathway are introduced with a vector containing a DHFR gene (for example, pCHOI) to compensate for the defect, and the copy number is amplified using methotrexate (MTX). Alternatively, a COS cell, which carries an SV40 T antigen-expressing gene on its chromosome, can be transformed with a vector containing the SV40 replication origin (for example, pcD) for transient gene expression. The replication origin may be derived from polyoma virus, adenovirus, bovine papilloma virus (BPV), and such. Furthermore, to increase the gene copy number in host cells, the expression vector may contain, as a selection marker, aminoglycoside transferase (APH) gene, thymidine kinase (TK) gene, *E. coli* xanthine guanine phosphoribosyl transferase (Ecogpt) gene, dihydrofolate reductase (dhfr) gene, and such.

[0089]

Next, the vector is introduced into a host cell. The host cells into which the vector is introduced are not particularly limited, for example, *E. coli* and various animal cells are available for this purpose. The host cells may be used, for example, as a production system to produce and express the antibodies of the present invention. *In vitro* and *in vivo* production systems are available for polypeptide production systems. Production systems that use eukaryotic cells or prokaryotic cells are examples of *in vitro* production systems.

[0090]

Eukaryotic cells that can be used are, for example, animal cells, plant cells, and fungal cells. Known animal cells include: mammalian cells, for example, CHO (J. Exp. Med. (1995)

108: 945), COS, 3T3, myeloma, BHK (baby hamster kidney), HeLa, Vero, amphibian cells such as *Xenopus laevis* oocytes (Valle, *et al.,* Nature (1981) 291: 358-340), or insect cells (e.g., Sf9, Sf21, and Tn5). In the present invention, CHO-DG44, CHO-DXB11, COS7 cells, and BHK cells can be suitably used. Among animal cells, CHO cells are particularly favorable for large-scale expression. Vectors can be introduced into a host cell by, for example, calcium phosphate methods, the DEAE-dextran methods, methods using cationic liposome DOTAP (Boehringer-Mannheim), electroporation methods, lipofection methods.

[0091]

Plant cells include, for example, *Nicotiana tabacum*-derived cells known as a protein production system. Calluses may be cultured from these cells. Known fungal cells include yeast cells, for example, genus *Saccharomyces* such as *Saccharomyces cerevisiae* and *Saccharomyces pombe*; and filamentous fungi, for example, genus *Aspergillus* such as *Aspergillus niger*.

[0092]

Bacterial cells can be used in the prokaryotic production systems. Examples of bacterial cells include *E. coli* (for example, JM109, DH5α, HB101 and such); and *Bacillus subtilis*.

[0093]

Next, the above host cells are cultured. Antibodies can be obtained by transforming the cells with a polynucleotide of interest and *in vitro* culturing of these transformants. Transformants can be cultured using known methods. For example, DMEM, MEM, RPMI 1640, or IMDM may be used as the culture medium for animal cells, and may be used with or without serum supplements such as FBS or fetal calf serum (FCS). Serum-free cultures are also acceptable. The preferred pH is about 6 to 8 during the course of culturing. Incubation is carried out typically at a temperature of about 30 to 40°C for about 15 to 200 hours. Medium is exchanged, aerated, or agitated, as necessary.

[0094]

On the other hand, production systems using animal or plant hosts may be used as systems for producing polypeptides *in vivo*. For example, a polynucleotide of interest is introduced into an animal or plant and the polypeptide is produced in the body of the animal or plant and then recovered. The "hosts" of the present invention includes such animals and plants.

[0095]

Animals to be used for the production system include mammals or insects. Mammals such as goats, pigs, sheep, mice, and cattle may be used (Vicki Glaser SPECTRUM Biotechnology Applications (1993)). Alternatively, the mammals may be transgenic animals.

For example, a polynucleotide of interest is prepared as a fusion gene with a gene encoding a polypeptide specifically produced in milk, such as the goat β-casein gene. DNA fragments containing the fusion gene are injected into goat embryos, which are then introduced back to female goats. The desired antibody can be obtained from milk produced by the transgenic goats, which are born from the goats that received the embryos, or from their offspring. Appropriate hormones may be administered to increase the volume of milk containing the antibody produced by the transgenic goats (Ebert, K.M. *et al.*, Bio/Technology (1994) 12: 699-702).

[0096]

Insects, such as silkworms, may also be used. Baculoviruses carrying a polynucleotide encoding an antibody of interest can be used to infect silkworms, and the antibody of interest can be obtained from the body fluids (Susumu, M. *et al.*, Nature (1985) 315: 592-594).

[0097]

Plants used in the production system include, for example, tobacco. When tobacco is used, a polynucleotide encoding an antibody of interest is inserted into a plant expression vector, for example, pMON 530, and then the vector is introduced into a bacterium, such as *Agrobacterium tumefaciens*. The bacteria are then used to infect tobacco such as *Nicotiana tabacum*, and the desired antibodies can be recovered from the leaves (Julian K.-C. Ma *et al.*, Eur. J. Immunol. (1994) 24: 131-138).

[0098]

The resulting antibody may be isolated from the inside or outside (such as the medium) of host cells, and purified as a substantially pure and homogenous antibody. Methods are not limited to any specific method and any standard method for isolating and purifying antibodies may be used. Polypeptides may be isolated and purified, by selecting an appropriate combination of, for example, chromatographic columns, filtration, ultrafiltration, salting out, solvent precipitation, solvent extraction, distillation, immunoprecipitation, SDS-polyacrylamide gel electrophoresis, isoelectric focusing, dialysis, recrystallization, and others.

[0099]

Chromatographies include, for example, affinity chromatographies, ion exchange chromatographies, hydrophobic chromatographies, gel filtrations, reverse-phase chromatographies, and adsorption chromatographies (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak *et al.*, Cold Spring Harbor Laboratory Press, 1996). These chromatographies can be carried out using liquid phase chromatographies such as HPLC and FPLC. Examples of the affinity chromatography columns include protein A columns and protein G columns. Examples of the proteins A columns include Hyper D, POROS, and Sepharose F. F. (Pharmacia).

[0100]

An antibody can be modified freely and peptide portions deleted by treating the antibody with an appropriate protein modifying enzyme before or after antibody purification. Such protein modifying enzymes include, for example, trypsins, chymotrypsins, lysyl endopeptidases, protein kinases, and glucosidases.

[0101]

Antibodies that bind to Mpl can be prepared by methods known to those skilled in the art.

For example, monoclonal antibody-producing hybridomas can be essentially generated by known technologies as follows: immunizing animals with Mpl proteins or Mpl-expressing cells as sensitized antigens using conventional immunological methods; fusing the obtained immunocytes with known parental cells by conventional cell fusion methods; and screening for monoclonal antibody-producing cells by conventional methods.

[0102]

Specifically, monoclonal antibodies can be prepared by the method below.

First, Mpl protein, which is used as a sensitized antigen for preparing antibodies, is prepared by expressing the Mpl gene/amino acid sequence (GenBank accession number: NM_005373). More specifically, the gene sequence encoding Mpl is inserted into a known expression vector, which is then transfected into an appropriate host cell. The subject human Mpl protein is purified from the host cell or culture supernatant using known methods.

[0103]

The purified Mpl protein is then used as a sensitized antigen. Alternatively, a partial Mpl peptide may be used as a sensitized antigen. In this case, the partial peptide can also be chemically synthesized based on the amino acid sequence of human Mpl.

The epitopes of Mpl molecule that are recognized by an anti-Mpl antibody of the present invention are not limited to a particular epitope, and may be any epitope on the Mpl molecule. Thus, any fragment can be used as an antigen for preparing anti-Mpl antibodies of the present invention, as long as the fragment comprises an epitope of the Mpl molecule.

[0104]

There is no limitation as to the type of mammalian species to be immunized with the sensitized antigen. However, a mammal is preferably selected based on its compatibility with the parental cell to be used in cell fusion. Generally, rodents (for example, mice, rats, and hamsters), rabbits, and monkeys can be used.

[0105]

Animals can be immunized with a sensitized antigen by known methods such as a routine method of injecting a sensitized antigen into a mammal intraperitoneally or

subcutaneously. Specifically, the sensitized antigen is diluted appropriately with phosphate-buffered saline (PBS), physiological saline and such, and then suspended. An adequate amount of a conventional adjuvant, for example, Freund's complete adjuvant, is mixed with the suspension, as necessary. An emulsion is then prepared for administering to a mammal several times over a 4- to 21-day interval. An appropriate carrier may be used for the sensitized antigen in immunization.

[0106]

A mammal is immunized as described above. After a titer increase of target antibody in the serum is confirmed, immunocytes are collected from the mammal and then subjected to cell fusion. Spleen cells are the preferred immunocytes.

[0107]

Mammalian myeloma cells are used as the parental cells to be fused with the above immunocytes. Preferable myeloma cells to be used include various known cell lines, for example, P3 (P3x63Ag8.653) (Kearney JF, *et al.*, J. Immnol. (1979) 123: 1548-1550), P3x63Ag8U.1 (Yelton DE, *et al.*, Current Topics in Microbiology and Immunology (1978) 81: 1-7), NS-1 (Kohler, G. and Milstein, C. Eur. J. Immunol. (1976) 6: 511-519), MPC-11 (Margulies, D. H. *et al.*, Cell (1976) 8: 405-415), SP2/0 (Shulman, M. *et al.*, Nature (1978) 276: 269-270), FO (deSt. Groth, S. F. *et al.*, J. Immunol. Methods (1980) 35: 1-21), S194 (Trowbridge, I. S., J. Exp. Med. (1978) 148: 313-323), and R210 (Galfre, G. *et al.*, Nature (1979) 277: 131-133).

[0108]

Cell fusions between the immunocytes and the myeloma cells as described above can be essentially carried out using known methods, for example, a method by Kohler and Milstein (Kohler, G. and Milstein, C., Methods Enzymol. (1981) 73: 3-46).

More specifically, the above-described cell fusions are carried out, for example, in a conventional culture medium in the presence of a cell fusion-promoting agent. The fusion-promoting agents include, for example, polyethylene glycol (PEG) and Sendai virus (HVJ). If required, an auxiliary substance such as dimethyl sulfoxide may also be added to improve fusion efficiency.

[0109]

The ratio of immunocytes to myeloma cells may be determined at one's own discretion, preferably, for example, one myeloma cell for every one to ten immunocytes. Culture media to be used for the above cell fusions include, for example, media that are suitable for the growth of the above myeloma cell lines, such as RPMI 1640 media and MEM media, and other conventional culture media used for this type of cell culture. In addition, serum supplements such as fetal calf serum (FCS) may also be used in combination.

[0110]

Cell fusion is carried out as follows. As described above, predetermined amounts of immunocytes and myeloma cells are mixed well in the culture medium. PEG solution (for example, mean molecular weight of about 1,000-6,000) pre-heated to 37°C is added to the cell suspension typically at a concentration of 30% to 60% (w/v), and mixed to produce fused cells (hybridomas). Then, an appropriate culture medium is successively added to the mixture, and the sample is centrifuged to remove supernatant. This treatment is repeated several times to remove the unwanted cell fusion-promoting agent and others that are unfavorable to hybridoma growth.

[0111]

Screening of the resulting hybridomas can be carried out by culturing them in a conventional selective medium, for example, hypoxanthine, aminopterin, and thymidine (HAT) medium. Culturing in the above-descried HAT medium is continued for a period long enough (typically, for several days to several weeks) to kill cells (non-fused cells) other than the desired hybridomas. Then, hybridomas are screened for single-cell clones capable of producing the target antibody by conventional limiting dilution methods.

[0112]

In addition to the method for preparing the above-descried hybridomas by immunizing non-human animals with antigens, preferred human antibodies having binding activity to Mpl can also be obtained by: sensitizing human lymphocytes with Mpl *in vitro*; and fusing the sensitized lymphocytes with human myeloma cells capable of dividing permanently (see, Japanese Patent Application Kokoku Publication No. (JP-B) H01-59878 (examined, approved Japanese patent application published for opposition)). Alternatively, it is possible to obtain human antibodies against Mpl from immortalized cells producing anti-Mpl antibodies. In this method, the cells producing anti-Mpl antibodies are prepared by administering Mpl as an antigen to transgenic animals comprising a repertoire of the entire human antibody genes (see, WO 94/25585, WO 93/12227, WO 92/03918, and WO 94/02602).

[0113]

The monoclonal antibody-producing hybridomas thus prepared can be passaged in a conventional culture medium, and stored in liquid nitrogen over long periods of time.

Monoclonal antibodies can be prepared from the above-described hybridomas by, for example, a routine procedure of culturing the hybridomas and obtaining antibodies from the culture supernatants. Alternatively, monoclonal antibodies can be prepared by injecting the hybridomas into a compatible mammal; growing these hybridomas in the mammal; and obtaining antibodies from the mammal's ascites. The former method is suitable for preparing highly purified antibodies, while the latter is suitable for preparing antibodies on a large scale.

[0114]

Recombinant antibodies can also be prepared by: cloning an antibody gene from a hybridoma; inserting the gene into an appropriate vector; introducing the vector into a host; and producing the antibodies by using genetic recombination techniques (see, for example, Vandamme, A. M. *et al.*, Eur. J. Biochem. (1990) 192: 767-775).

[0115]

Specifically, an mRNA encoding the variable (V) region of anti-Mpl antibody is isolated from hybridomas producing the anti-Mpl antibodies. For mRNA isolation, total RNAs are first prepared by conventional methods such as guanidine ultracentrifugation methods (Chirgwin, J. M. *et al.*, Biochemistry (1979) 18: 5294-5299), or acid guanidinium thiocyanate-phenol-chloroform (AGPC) methods (Chomczynski, P. *et al.*, Anal. Biochem. (1987) 162: 156-159), and then the target mRNA is prepared using an mRNA Purification Kit (Pharmacia) and such. Alternatively, the mRNA can be directly prepared using the QuickPrep mRNA Purification Kit (Pharmacia).

[0116]

A cDNA of the antibody V region is synthesized from the resulting mRNA using reverse transcriptase. cDNA synthesis is carried out using the AMV Reverse Transcriptase First-strand cDNA Synthesis Kit (Seikagaku Co.), or such. Alternatively, cDNA can be synthesized and amplified by the 5'-RACE method (Frohman, M. A. *et al.*, Proc. Natl. Acad. Sci. USA (1988) 85: 8998-9002; Belyavsky, A. *et al.*, Nucleic Acids Res. (1989) 17: 2919-2932) using the 5'-Ampli FINDER RACE Kit (Clontech) and PCR.

[0117]

Target DNA fragments are purified from the obtained PCR products and then ligated with vector DNAs to prepare recombinant vectors. The vectors are introduced into *E. coli* and such, and colonies are selected for preparing the recombinant vector of interest. The target DNA nucleotide sequence is then confirmed by conventional methods such as the dideoxynucleotide chain termination method.

Once a DNA encoding the V region of target anti-Mpl antibody is obtained, the DNA is inserted into an expression vector which comprises a DNA encoding the constant region (C region) of a desired antibody.

[0118]

The method for producing anti-Mpl antibodies to be used in the present invention typically comprises the steps of: inserting an antibody gene into an expression vector, so that the gene is expressed under the regulation of expression regulatory regions, such as enhancer and promotor; and transforming host cells with the resulting vectors to express antibodies.

[0119]

For expressing the antibody gene, polynucleotides encoding H chain and L chain,

respectively, are inserted into separate expression vectors and co-transfected into a host cell. Alternatively, polynucleotides encoding both H chain and L chain are inserted into a single expression vector and transfected into a host cell (see WO 94/11523).

[0120]

The term "agonistic activity" refers to an activity to induce changes in some biological activities through signal transduction into cells and such, due to the binding of an antibody to a receptor antigen. The biological activities include, for example, proliferation-promoting activities, proliferation activities, viability activities, differentiation-inducing activities, differentiation activities, transcriptional activities, membrane transport activities, binding activities, proteolytic activities, phosphorylation/dephosphorylation activities, oxidation/reduction activities, transfer activities, nucleolytic activities, dehydration activities, cell death-inducing activities, and apoptosis-inducing activities, but is not limited thereto.

[0121]

The term "agonistic activity against Mpl" typically refers to the activity of promoting the differentiation of megakaryocytes or their parental hemopoietic stem cells into platelets, or the activity of stimulating platelet proliferation.

Agonistic activity can be assayed by methods known to those skilled in the art. The agonistic activity may be determined using the original activity or a different activity as an indicator.

[0122]

For example, agonistic activity can be determined by a method using cell growth as an indicator as described in Examples. More specifically, an antibody whose agonistic activity is to be determined is added to cells which proliferate in an agonist-dependent manner, followed by incubation of the cells. Then, a reagent such as WST-8, which shows a coloring reaction at specific wavelengths depending on the viable cell count, is added to the culture and absorbance is measured. The agonistic activity can be determined using the measured absorbance as an indicator.

[0123]

Cells that proliferate in an agonist-dependent manner can also be prepared by methods known to those skilled in the art. For example, when the antigen is a receptor capable of transducing cell growth signals, cells expressing the receptor may be used. Alternatively, when the antigen is a receptor that cannot transduce signals, a chimeric receptor consisting of the intracellular domain of a receptor that transduces cell growth signals and the extracellular domain of a receptor that does not transduce cell growth signals can be prepared for cellular expression. Receptors that transduce cell growth signals include, for example, G-CSF receptors, mpl, neu, GM-CSF receptors, EPO receptors, c-kit, and FLT-3. Cells that can be used to

express a receptor include, for example, BaF3, NFS60, FDCP-1, FDCP-2, CTLL-2, DA-1, and KT-3.

[0124]

There is no limitation as to the type of detection indicators to be used for determining agonistic activity, as long as the indicator can monitor quantitative and/or qualitative changes. For example, it is possible to use cell-free assay indicators, cell-based assay indicators, tissue-based assay indicators, and *in vivo* assay indicators. Indicators that can be used in cell-free assays include enzymatic reactions, quantitative and/or qualitative changes in proteins, DNAs, or RNAs. Such enzymatic reactions include, for example, amino acid transfers, sugar transfers, dehydrations, dehydrogenations, and substrate cleavages. Alternatively, protein phosphorylations, dephosphorylations, dimerizations, multimerizations, hydrolyses, dissociations and such; DNA or RNA amplifications, cleavages, and extensions can be used as the indicator in cell-free assays. For example, protein phosphorylations downstream of a signal transduction pathway may be used as a detection indicator. Alterations in cell phenotype, for example, quantitative and/or qualitative alterations in products, alterations in growth activity, alterations in cell number, morphological alterations, or alterations in cellular properties, can be used as the indicator in cell-based assays. The products include, for example, secretory proteins, surface antigens, intracellular proteins, and mRNAs. The morphological alterations include, for example, alterations in dendrite formation and/or dendrite number, alteration in cell flatness, alteration in cell elongation/axial ratio, alterations in cell size, alterations in intracellular structure, heterogeneity/homogeneity of cell populations, and alterations in cell density. Such morphological alterations can be observed under a microscope. Cellular properties to be used as the indicator include anchor dependency, cytokine-dependent response, hormone dependency, drug resistance, cell motility, cell migration activity, pulsatory activity, and alteration in intracellular substances. Cell motility includes cell infiltration activity and cell migration activity. The alterations in intracellular substances include, for example, alterations in enzyme activity, mRNA levels, levels of intracellular signaling molecules such as Ca^{2+} and cAMP, and intracellular protein levels. When a cell membrane receptor is used, alterations in the cell proliferating activity induced by receptor stimulation can be used as the indicator. The indicators to be used in tissue-based assays include functional alterations adequate for the subject tissue. In *in vivo* assays, alterations in tissue weight, alterations in the blood system (for example, alterations in blood cell counts, protein contents, or enzyme activities), alterations in electrolyte levels, and alterations in the circulating system (for example, alterations in blood pressure or heart rate).

[0125]

The methods for measuring such detection indices are not particularly limited. For

example, absorbance, luminescence, color development, fluorescence, radioactivity, fluorescence polarization, surface plasmon resonance signal, time-resolved fluorescence, mass, absorption spectrum, light scattering, and fluorescence resonance energy transfer may be used. These measurement methods are known to those skilled in the art and may be selected appropriately depending on the purpose. For example, absorption spectra can be obtained by using a conventional photometer, plate reader, or such; luminescence can be measured with a luminometer or such; and fluorescence can be measured with a fluorometer or such. Mass can be determined with a mass spectrometer. Radioactivity can be determined with a device such as a gamma counter depending on the type of radiation. Fluorescence polarization can be measured with BEACON (TaKaRa). Surface plasmon resonance signals can be obtained with BIACORE. Time-resolved fluorescence, fluorescence resonance energy transfer, or such can be measured with ARVO or such. Furthermore, a flow cytometer can also be used for measuring. It is possible to use one of the above methods to measure two or more different types of detection indices. A greater number of detection indices may also be examined by using two or more measurement methods simultaneously and/or consecutively. For example, fluorescence and fluorescence resonance energy transfer can be measured at the same time with a fluorometer.

[0126]

The present invention provides pharmaceutical compositions comprising antibodies of this invention. The pharmaceutical compositions comprising antibodies of the present invention are useful for treating and/or preventing thrombocytopenia and such. Time required for the platelet count to recover to the normal level can be shortened by administering an antibody of the present invention after donation of platelet components. The amount of platelet components at the time of blood collection can be increased by pre-administering an antibody of the present invention.

[0127]

When used as pharmaceutical compositions, the antibodies of the present invention can be formulated by methods known to those skilled in the art. For example, the antibodies can be administered parenterally by injection of a sterile solution or suspension in water or other pharmaceutically acceptable solvents. For example, the antibodies can be formulated by appropriately combining with pharmaceutically-acceptable carriers or solvents, specifically, sterile water or physiological saline, vegetable oils, emulsifiers, suspending agents, surfactants, stabilizers, flavoring agents, excipients, vehicles, preservatives, binding agents, and such, and mixing at a unit dosage and form required by accepted pharmaceutical implementations. In such formulations, the amount of the thus obtained active ingredient should be within the required range.

[0128]

A sterile composition to be injected can be formulated using a vehicle such as distilled water used for injection, according to standard protocols.

Aqueous solutions used for injections include, for example, physiological saline and isotonic solutions comprising glucose or other adjunctive agents such as D-sorbitol, D-mannose, D-mannitol, and sodium chloride. They may also be combined with an appropriate solubilizing agent such as alcohol, specifically, ethanol, polyalcohol such as propylene glycol or polyethylene glycol, or non-ionic detergent such as polysorbate 80™ or HCO-50, as necessary.

[0129]

Oil solutions include sesame oils and soybean oils, and can be combined with solubilizing agents such as benzyl benzoate or benzyl alcohol. Injection solutions may also be formulated with buffers, for example, phosphate buffers or sodium acetate buffers; analgesics, for example, procaine hydrochloride; stabilizers, for example, benzyl alcohol or phenol; or anti-oxidants. The prepared injections are typically aliquoted into appropriate ampules.

[0130]

The administration is preferably carried out parenterally, specifically, by injection, intranasal administration, intrapulmonary administration, percutaneous administration, or such. Injections include, for example, intravenous injections, intramuscular injections, intraperitoneal injections, and subcutaneous injections. The injection solutions can be also administered systemically or locally.

[0131]

The administration methods can be selected properly according to the patient's age, condition, and such. The applied dose of a pharmaceutical composition comprising an antibody or polynucleotide encoding the antibody may be, for example, in the range of 0.0001 to 1,000 mg/kg body weight. Alternatively, the dosage may be, for example, in the range of 0.001 to 100,000 mg/kg body weight. However, the dosage is not restricted to the values described above. The dosage and administration methods depend on the patient's weight, age, and condition, and are appropriately selected by those skilled in the art.

[Examples]

[0132]

The present invention is specifically illustrated below with reference to Examples, but it is not to be construed as being limited thereto.

[Example 1] Preparation of anti-human Mpl antibodies

1.1 Establishment of Mpl-expressing BaF3 cell lines

BaF3 cell lines expressing the full-length Mpl gene were established to obtain cell lines that proliferate in a TPO-dependent manner.

A full-length human Mpl cDNA (Palacios, R. *et al.*, Cell (1985) 41: 727-734) (GenBank accession NO. NM_005373) was amplified by PCR. The cDNA was cloned into a pCOS2 expression vector to construct pCOS2-hMplfull. The expression vector pCOS2 was constructed by removing the DHFR gene expression region from pCHOI (Hirata, Y. *et al.*, FEBS Letter (1994) 356: 244-248), where the expression region of the neomycin resistance gene HEF-VH-gγ1 (Sato, K. *et al.*, Mol Immunol. (1994) 31: 371-381) is inserted.

The cynomolgus monkey Mpl cDNA (SEQ ID NO: 164) was cloned from total RNA extracted from the bone marrow cells of cynomolgus monkey, using a SMART RACE cDNA Amplification Kit (Clontech). The resulting cynomolgus monkey cDNA was inserted into pCOS2 to construct pCOS2-monkeyMplfull.

Then, the full-length mouse Mpl cDNA (GenBank accession NO. NM_010823) was amplified by PCR, and inserted into pCOS2 to construct pCOS2-mouseMplfull.

[0133]

Each vector (20 μg) prepared as described above was mixed with BaF3 cells (1 x 10^7 cells/mL) suspended in PBS in Gene Pulser cuvettes. This mixture was then pulsed at 0.33 kV and 950 μFD using a Gene Pulser II (Bio-Rad). The BaF3 cells introduced with the above DNAs by electroporation were added to RPMI 1640 medium (Invitrogen) containing 1 ng/mL mouse interleukin 3 (hereinafter abbreviated as mIL-3; Peprotech), 500 μg/mL Geneticin (Invitrogen), and 10% FBS (Invitrogen), and selected to establish a human Mpl-expressing BaF3 cell line (hereinafter abbreviated as "BaF3-human Mpl"), monkey Mpl-expressing BaF3 cell line (hereinafter abbreviated as BaF3-monkey Mpl), and mouse Mpl-expressing BaF3 cell line (hereinafter abbreviated as "BaF3-mouse Mpl"). Following selection, these cells were cultured and maintained in RPMI 1640 containing 1 ng/mL rhTPO (R&D) and 10% FBS.

[0134]

1.2 Establishment of Mpl-expressing CHO cell lines

CHO cell lines expressing the full-length Mpl gene were established to obtain cell lines to be used for assessing binding activity by flow cytometry.

First, the DHFR gene expression site from pCHOI was inserted into pCXN2 (Niwa, H. *et al.*, Gene (1991) 108: 193-199) at the *Hind*III site to prepare a pCXND3expression vector. The respective Mpl genes were amplified by PCR using pCOS2-hMplfull, pCOS2-monkeyMplfull, and pCOS2-mouseMplfull as templates, and primers with a His-tag sequence. The PCR products were cloned into pCXND3 to construct pCXND3-hMpl-His, pCXND3-monkey Mpl-His, and pCXND3-mouse Mpl-His, respectively.

[0135]

Vectors thus prepared (25 μg each) were mixed with a PBS suspension of CHO-DG44 cells (1 x 10^7 cells/mL) in Gene Pulser cuvettes. The mixture was then pulsed at 1.5 kV and 25

μFD using Gene Pulser II (Bio-Rad). The CHO cells introduced with these DNAs by electroporation were added to CHO-S-SFMII medium (Invitrogen) containing 500 μg/mL Geneticin and 1x HT (Invitrogen). A human Mpl-expressing CHO cell line (hereinafter abbreviated as "CHO-human Mpl"), monkey Mpl-expressing CHO cell line (hereinafter abbreviated as "CHO-monkey Mpl"), and mouse Mpl-expressing CHO cell line (hereinafter abbreviated as "CHO-mouse Mpl") were established through selection.

[0136]

1.3 Preparation of soluble human Mpl protein

To prepare soluble human Mpl protein, an expression system using insect Sf9 cells for production and secretion of the protein was constructed as described below.

A DNA construct encoding the extracellular region of human Mpl (Gln 26 to Trp 491) with a downstream FLAG tag was prepared. The construct was inserted into a pBACSurf-1 Transfer Plasmid (Novagen) between the *Pst*I and *Sma*I sites to prepare pBACSurf1-hMpl-FLAG. Then, Sf9 cells were transformed with 4 μg of pBACSurf1-hMpl-FLAG using the Bac-N-Blue Transfection Kit (Invitrogen). The culture supernatant was collected after three-day incubation. Recombinant virus was isolated by plaque assays. The prepared virus stock was used to infect Sf9 cells, and the culture supernatant was collected.

[0137]

Soluble human Mpl protein was purified from the obtained culture supernatant as described below. The culture supernatant was loaded onto a Q Sepharose Fast Flow (Amersham Biosciences) for adsorption, and the adsorbed protein was then eluted with 50 mM Na-phosphate buffer (pH7.2) containing 0.01% (v/v) Tween20 and 500 mM NaCl. After the eluates were loaded onto a FLAG M2-Agarose (Sigma-Aldrich) for adsorption, the protein adsorbed was eluted with 100 mM glycine-HCl buffer (pH3.5) containing 0.01% (v/v) Tween20. Immediately after elution, the fraction obtained was neutralized with 1 M Tris-Cl (pH8.0) and the buffer was exchanged with PBS (-) and 0.01% (v/v) Tween20 using PD-10 columns (Amersham Biosciences). The purified soluble Mpl protein was referred to as "shMpl-FLAG".

[0138]

1.4 Preparation of human Mpl-IgG Fc fusion protein

Human fusion protein Mpl-IgG Fc gene was prepared according to the method by Bennett *et al.* (Bennett, B. D. *et al.*, J. Biol. Chem. (1991) 266: 23060-23067). A nucleotide sequence encoding the extracellular region of human Mpl (Gln 26 to Trp 491) was linked to a nucleotide sequence encoding the Fc region of human IgG-γ1 (a region downstream of Asp 216). A *Bst*EII sequence (amino acids: Val-Thr) was attached to the junction as a fusion linker between these two regions. A 19-amino acid signal peptide derived form human IgG H chain variable region was used as the signal sequence. The resulting human fusion protein Mpl-IgG Fc gene

was cloned into pCXND3 to construct pCXND3-hMpl-Fc.

The vector thus prepared (25 μg) was mixed with a PBS suspension of CHO-DG44 cells (1×10^7 cells/mL) in Gene Pulser cuvettes. The mixture was then pulsed at 1.5 kV and 25 μFD using Gene Pulser II (Bio-Rad). The CHO cells introduced with the DNA by electroporation were added to CHO-S-SFMII medium containing 500 μg/mL Geneticin and 1x HT (Invitrogen). shMPL-Fc-expressing CHO cell line (CHO-hMpl-Fc) was then established through selection.

[0139]

Human Mpl-IgG Fc fusion protein was purified from the culture supernatant as described below.

The culture supernatant was loaded onto a Q Sepharose Fast Flow (Amersham Biosciences) for adsorption, and then the adsorbed protein were eluted with 50 mM Na-phosphate buffer (pH7.6) containing 0.01% (v/v) Tween20 and 1 M NaCl. After the eluates were loaded onto a HiTrap protein G HP column (Amersham Biosciences) for adsorption, the adsorbed protein was eluted with 0.1 M glycine-HCl buffer (pH2.7) containing 150 mM NaCl and 0.01% (v/v) Tween20. Immediately after elution, the obtained fraction was neutralized with 1 M Tris-Cl (pH8.0) and the buffer was exchanged with PBS (-) and 0.01% (v/v) Tween20 using PD-10 columns (Amersham Biosciences). The purified soluble Mpl protein was referred to as "hMpl-Fc".

[0140]

1.5 Immunization with shMpl-FLAG or BaF3-human Mpl and hybridoma selection

MRL/MpJUmmCrj-lpr/lpr mice (hereinafter abbreviated as "MRL/lpr mice"; purchased from Charles River, Japan) were immunized; the primary immunization was carried out at eight weeks of age. For every single mouse, an emulsion containing 100 μg of shMPL-FLAG combined with Freund's complete adjuvant (H37 Ra; Beckton Dickinson), was administered subcutaneously as the primary injection. As a booster injection, an emulsion containing shMPL-FLAG (50 μg per mouse) combined with Freund's incomplete adjuvant (Beckton Dickinson) was administered subcutaneously. Three mice which have been immunized six times in total were subjected to a final injection of shMPL-FLAG (50 μg per mouse) through the caudal vein. Cell fusion was achieved by mixing the mouse myeloma P3-X63Ag8U1 cells (P3U1; purchased from ATCC) and mouse splenocytes using polyethylene glycol 1500 (Roche Diagnostics). Hybridoma selection in HAT medium began the following day and culture supernatants were obtained. Screening was carried out by ELISA, using immunoplates immobilized with shMpl-FLAG or hMpl-Fc and the assayed cell growth activity of BaF3-human Mpl as an index. In addition, Balb/C mice were immunized eleven times in total by administering BaF3-human Mpl (1.0×10^7 cells per mouse) intraperitoneally over a period of one week to five months. Hybridomas were similarly prepared by cell fusion, and screened

using the assayed cell growth activity of BaF3-human Mpl as an index. Positive clones were isolated as single clones by limiting dilution and then cultured in a large scale. The culture supernatants were collected.

[0141]

1.6 Analyses of anti-human Mpl antibodies

Antibody concentrations were determined by carrying out a mouse IgG sandwich ELISA using goat anti-mouse IgG (gamma) (ZYMED) and alkaline phosphatase-goat anti-mouse IgG (gamma) (ZYMED), generating a calibration curve by GraphPad Prism (GraphPad Software; USA), and calculating the antibody concentrations from the calibration curve. Commercially available antibodies of the same isotype were used as standards.

Antibody isotypes were determined by antigen-dependent ELISA using isotype-specific secondary antibodies. hMpl-Fc was diluted to 1 μg/mL with a coating buffer (0.1 mM $NaHCO_3$, pH9.6) containing 0.02% (w/v) NaN_3, and then added to ELISA plates. The plates were incubated overnight at 4°C for coating. The plates were blocked with a diluent buffer (50 mM Tris-HCl (pH8.1) containing 1 mM $MgCl_2$, 150 mM NaCl, 0.05% (v/v) Tween20, 0.02% (w/v) NaN_3, 1% (w/v) BSA). After the addition of hybridoma culture supernatants, the plates were allowed to stand at room temperature for 1 hr. After washing with a rinse buffer (0.05% (v/v) Tween20 in PBS), alkaline phosphatase-labeled isotype-specific secondary antibodies were added to the plates. Then, the plates were allowed to stand at room temperature for 1 hr. Color development was carried out using SIGMA104 (Sigma-Aldrich) diluted to 1 mg/mL with a substrate buffer (50 mM $NaHCO_3$, pH9.8) containing 10 mM $MgCl_2$, and absorbance was measured at 405 nm using Benchmark Plus (Bio-Rad).

[0142]

The binding activities of an antibody to shMpl-FLAG and hMPL-Fc were determined by ELISA. ELISA plates were coated with 1 μg/mL of purified shMpl-FLAG or hMPL-Fc, and blocked with a diluent buffer. Hybridoma culture supernatants were added to the plates, and the plates were allowed to stand at room temperature for 1 hr. Then, alkaline phosphatase-labeled anti-mouse IgG antibodies (Zymed) were added to the plates. Color development was similarly carried out using the above method. Following a one-hour coloring reaction at room temperature, absorbance was measured at 405 nm and EC_{50} values were computed using GraphPad Prism.

CHO-human Mpl cells and CHO-monkey Mpl cells were harvested, and suspended in FACS Buffer (1% FBS/ PBS) to a final concentration of 1×10^6 cells/mL. The suspensions were aliquoted into Multiscreen (Millipore) at 100 μl/well, and the culture supernatants were removed by centrifugation. Culture supernatants diluted to 5 μg/mL were added to the plates and incubated on ice for 30 min. The cells were washed once with FACS buffer, and incubated

on ice for 30 min following the addition of an FITC-labeled anti-mouse IgG antibody (Beckman Coulter). After incubation, the mixture was centrifuged at 500 rpm for 1 min. The supernatants were removed, and then the cells were suspended in 400 μL of FACS buffer. The samples were analyzed by flow cytometry using EPICS ELITE ESP (Beckman Coulter). An analysis gate was set on the forward and side scatters of a histogram to include viable cell populations.

[0143]

Agonistic activities of an antibody were evaluated using BaF3-human Mpl and BaF3-monkey Mpl which proliferate in a TPO-dependent manner. Cells of each cell line were suspended at 4 x 10^5 cells/ml in RPMI 1640/10% FBS (Invitrogen), and each suspension was aliquoted into a 96-well plate at 60μl/well. A 40-μL aliquot of rhTPO (R&D) and hybridoma culture supernatants prepared at various concentrations was added into each well. The plates were then incubated at 37°C under 5% CO_2 for 24 hr. A 10-μL aliquot of the Cell Count Reagent SF (Nacalai Tesque) was added into each well. After incubation for 2 hr, absorbance was measured at 450 nm (and at 655 nm as a control) using a Benchmark Plus. EC_{50} values were calculated using GraphPad Prism.

The above analysis yielded a total of 163 clones of mouse monoclonal antibodies that bind to human Mpl.

Among the anti-human Mpl antibodies to be described, TA136 was established from mice immunized with BaF-human Mpl and the others were established from mice immunized with shMpl-Flag.

[0144]

1.7 Purification of anti-human Mpl antibodies

Anti-human Mpl antibodies were purified from hybridoma culture supernatants as described below.

After the culture supernatants were loaded onto HiTrap protein G HP columns (Amersham Biosciences) for adsorption, the antibodies were eluted with 0.1 M glycine-HCl (pH2.7). Immediately after elution, the fractions were neutralized with 1 M Tris-Cl (pH9.0), dialyzed against PBS for one day, and the buffer was replaced.

[0145]

1.8 Determination of epitopes for the anti-human Mpl antibody VB22B

Since the anti-human Mpl antibody VB22B can be used for Western blotting, a GST-fusion protein containing a partial sequence of human Mpl was constructed for VB22B epitope analysis. MG1 (Gln26 to Trp491) and MG2 (Gln26 to Leu274) regions were each amplified by PCR, and cloned into pGEX-4T-3 (Amersham Biosciences) to be expressed as GST fusion proteins. The resulting plasmid DNAs were transformed into DH5α to give

transformants. A final concentration of 1 mM IPTG was added to the transformants in their logarithmic growth phase to induce the expression of GST fusion proteins. The bacterial cells were harvested after two hours of incubation. The cells were lysed by sonication. The lysates were centrifuged in XL-80 Ultracentrifuge (Beckman, Rotor 70.1Ti) at 35,000 rpm for 30 min. The culture supernatants were removed, and then the fusion proteins were purified using GST Purification Modules (Amersham Biosciences). The samples were separated by 10%-SDS-PAGE, and then transferred onto a PVDF membrane. The membrane was Western blotted with the murine antibody VB22B. VB22B was found to recognize both MG-1 and MG-2, indicating that the VB22B epitope is located in the (Gln26 to Leu274) region.

[0146]

Then, GST fusion proteins containing the respective regions of human Mpl: MG3 (Gln26 to Ala189), MG4 (Gln26 to Pro106), MG5 (Gln26 to Glu259), and MG6 (Gln26 to Gly245) were prepared and analyzed by Western blotting using the same procedure described above. VB22B was found to recognize MG5 and MG6, but not MG3 and MG4. This suggests that the VB22B epitope is located within the (Ala189 to Gly245) region. In addition, GST was fused with MG7 (Gln26 to Ala231) and MG8 (Gln26 to Pro217) to prepare GST fusion proteins. VB22B recognized MG7 but not MG8, suggesting that the VB22B epitope is located in the (Gln217 to Ala231) region. Furthermore, GST fusion protein containing MG10 (Gln213 to Ala231) was recognized by VB22B, suggesting that the VB22B epitope is located within the limited region of 19 amino acids between Gln213 and Ala231.

[0147]

1.9 Kinetic analyses of the antigen-antibody reaction for anti-human Mpl antibody VB22B

Since the anti-human Mpl antibody VB22B binds to soluble recombinant Mpl, kinetic analyses of the antigen-antibody reaction between VB22B IgG and human Mpl-IgG Fc fusion protein were carried out as described in Example 1.4. The Sensor Chip CM5 (Biacore) was placed in Biacore 2000 (Biacore), and human Mpl-IgG Fc fusion protein was immobilized onto the chip by amine-coupling methods. Then, 1.25 to 20 μg/mL of VB22B IgG solution was prepared using HBS-EP Buffer (Biacore), and injected over the chip surface for 2 min to reveal the binding region. Then, HBS-EP Buffer was injected over the chip surface for 2 min to reveal the dissociation region. VB22B IgG bound to the human Mpl-IgG Fc fusion protein on the sensor chip was removed by injecting 10 mM NaOH over the sensor chip for 15 sec, and the chip was recovered. HBS-EP Buffer was used as the running buffer, and the flow rate was 20 μL/min. Using the BIAevaluation Version 3.1 (Biacore) software, the reaction rate constant at each concentration was calculated from the sensorgrams. The dissociation constant (KD) for VB22B IgG was determined to be $1.67 \pm 0.713 \times 10^{-9}$ M.

[0148]

[Example 2] Preparation of single-chain anti-human Mpl antibodies

Among the prepared anti-human Mpl antibodies, 23 types of antibodies, which exhibit higher binding activities and agonistic activities, were selected to construct expression systems for single-chain antibodies using genetic engineering techniques. An exemplary method for constructing a single-chain antibody derived from the anti-human Mpl antibody VB22B is described below.

[0149]

2.1 Cloning of the anti-human Mpl antibody variable region

The variable region was amplified by RT-PCR using total RNA extracted from hybridomas producing anti-human Mpl antibodies. Total RNA was extracted from 1 x 10^7 hybridoma cells using the RNeasy Plant Mini Kit (QIAGEN).

A 5'-terminal fragment of the gene was amplified from 1 μg of total RNA by the SMART RACE cDNA Amplification Kit (Clontech), using a synthetic oligonucleotide MHC-IgG2b (SEQ ID NO: 166) complementary to mouse IgG2b constant region or a synthetic oligonucleotide kappa (SEQ ID NO: 167) complementary to mouse κ chain constant region. Reverse transcription was carried out at 42°C for 1.5 hr.

[0150]

The composition of the PCR reaction solution (50 μL in total) is shown below.

10x Advantage 2 PCR Buffer (Clontech)	5 μL
10x Universal Primer A Mix (Clontech)	5 μL
dNTPs (dATP, dGTP, dCTP, and dTTP) (Clontech)	0.2 mM
Advantage 2 Polymerase Mix (Clontech)	1 μL
Reverse transcription product	2.5 μL
Synthetic oligonucleotide, MHC-IgG2b or kappa	10 pmol

The PCR reaction conditions were:

94°C (initial temperature) for 30 sec;
five cycles of 94°C for 5 sec and 72°C for 3 min;
five cycles of 94°C for 5 sec, 70°C for 10 sec, and 72°C for 3 min;
25 cycles of 94°C for 5 sec, 68°C for 10 sec, and 72°C for 3 min;
and final extension was at 72°C for 7 min.

[0151]

The PCR products were purified from agarose gel using the QIAquick Gel Extraction Kit (QIAGEN), and cloned into a pGEM-T Easy Vector (Promega). The nucleotide sequence was then determined using the ABI 3700 DNA Analyzer (Perkin Elmer).

The nucleotide sequence of cloned VB22B H chain variable region (hereinafter abbreviated as "VB22B-VH") is shown in SEQ ID NO: 117, and its amino acid sequence is

shown in SEQ ID NO: 118. The nucleotide sequence of the L chain variable region (hereinafter abbreviated as "VB22B-VL") is shown in SEQ ID NO: 119, and its amino acid sequence is shown in SEQ ID NO: 120.

[0152]

2.2 Preparation of expression vectors for anti-human Mpl diabodies

The gene encoding VB22B single-chain Fv (hereinafter abbreviated as "VB22B diabody") containing a five-amino acid linker sequence was constructed, by linking a nucleotide sequence encoding a $(Gly4Ser)_1$ linker to the VB22B-VH-encoding gene at its 3' end and to the VB22B-VL-encoding gene at its 5' end; both of which have been amplified by PCR.

[0153]

The VB22B-VH forward primer, 70·115HF, (SEQ ID NO: 168) was designed to contain an *Eco*RI site. The VB22B-VH reverse primer, 33·115HR, (SEQ ID NO: 169) was designed to hybridize to a DNA encoding the C terminus of VB22B-VH, and to have a nucleotide sequence encoding the $(Gly4Ser)_1$ linker and a nucleotide sequence hybridizing to the DNA encoding the N terminus of VB22B-VL. The VB22B-VL forward primer, 33·115LF, (SEQ ID NO: 170) was designed to have a nucleotide sequence encoding the N terminus of VB22B-VL, a nucleotide sequence encoding the $(Gly4Ser)_1$ linker, and a nucleotide sequence encoding the C terminus of VB22B-VH. The VB22B-VL reverse primer, 33·115LR, (SEQ ID NO: 171) was designed to hybridize to a DNA encoding the C terminus of VB22B-VL and to have a nucleotide sequence encoding a FLAG tag (Asp Tyr Lys Asp Asp Asp Asp Lys/SEQ ID NO: 172) and a *Not*I site.

[0154]

In the first round of PCR, two PCR products: one containing VB22B-VH and a linker sequence, and the other containing VB22B-VL and the identical linker sequence, were synthesized by the procedure described below.

The composition of the PCR reaction solution (50 μL in total) is shown below.

10x PCR Buffer (TaKaRa)	5 μL
dNTPs (dATP, dGTP, dCTP, and dTTP) (TaKaRa)	0.4 mM
DNA polymerase TaKaRa Ex Taq (TaKaRa)	2.5 units
pGEM-T Easy vector comprising VB22B-VH or VB22B-VL gene	10 ng
Synthetic oligonucleotides, 70·115HF and 33·115HR, or 33·115LF and 33·115LR	10 pmol

The PCR reaction conditions were:

94°C (initial temperature) for 30 sec;

five cycles of: 94°C for 15 sec and 72°C for 2 min;

five cycles of 94°C for 15 sec and 70°C for 2 min;

28 cycles of 94°C for 15 sec and 68°C for 2 min;

and final extension was at 72°C for 5 min.

[0155]

After the PCR products of about 400 bp were purified from agarose gel using the QIAquick Gel Extraction Kit (QIAGEN), the second-round PCR was carried out using aliquots of the respective PCR products according to the protocol described below.

The composition of the PCR reaction solution (50 μL in total) is shown below.

10x PCR Buffer (TaKaRa)	5 μL
dNTPs (dATP, dGTP, dCTP, and dTTP) (TaKaRa)	0.4 mM
DNA polymerase TaKaRa Ex Taq (TaKaRa)	2.5 unit
First-round PCR products (two types)	1 μL
Synthetic oligonucleotides, 70·115HF and 33·115LR	10 pmol

The reaction conditions were:

94°C (initial temperature) for 30 sec;

five cycles of 94°C for 15 sec and 72°C for 2 min;

five cycles of 94°C for 15 sec and 70°C for 2 min;

28 cycles of 94°C for 15 sec and 68°C for 2 min;

and final extension was at 72°C for 5 min.

[0156]

The PCR products of about 800 bp were purified from agarose gel using the QIAquick Gel Extraction Kit (QIAGEN), and then digested with *Eco*RI and *Not*I (both from TaKaRa). The resulting DNA fragments were purified using the QIAquick PCR Purification Kit (QIAGEN), and then cloned into pCXND3 to prepare pCXND3-VB22B db.

[0157]

2.3 Preparation of expression vectors for anti-human Mpl antibody sc(Fv)$_2$

To prepare expression plasmids for the modified antibody [sc(Fv)$_2$] comprising two units of H chain variable region and two units of L chain variable region derived from VB22B, the above-described pCXND3-VB22B db was modified by PCR using the procedure shown below. The process for constructing the sc(Fv)$_2$ gene is illustrated in Fig. 1.

[0158]

First, PCR method was carried out to amplify (a) the VB22B-VH-encoding gene in which a nucleotide sequence encoding a 15-amino acid linker (Gly$_4$Ser)$_3$ was added to its 3' end; and (b) the VB22B-VL-encoding gene containing the identical linker nucleotide sequence added to its 5' end. The desired construct was prepared by linking these amplified genes. Three new primers were designed in this construction process. The VB22B-VH forward primer, VB22B-fpvu, (primer A; SEQ ID NO: 173) was designed to have an *Eco*RI site at its 5' end and to convert Gln22 and Leu23 of VB22B db into a *Pvu*II site. The VB22B-VH reverse primer,

sc-rL15, (primer B; SEQ ID NO: 174) was designed to hybridize to a DNA encoding the C terminus of VB22B-VH, and to have a nucleotide sequence encoding the $(Gly4Ser)_3$ linker, as well as a nucleotide sequence hybridizing to a DNA encoding the N terminus of VB22B-VL. The VB22B-VL forward primer, sc-fL15, (primer C; SEQ ID NO: 175) was designed to have a nucleotide sequence encoding the N terminus of VB22B-VL, a nucleotide sequence encoding the $(Gly_4Ser)_3$ linker, and a nucleotide sequence encoding the C terminus of VB22B-VH.

[0159]

In the first-round PCR, two PCR products: one comprising VB22B-VH and a linker sequence, and the other comprising VB22B-VL and the identical linker sequence, were synthesized by the procedure described below.

The composition of the PCR reaction solution (50 μL in total) is shown below.

10x PCR Buffer (TaKaRa)	5 μL
dNTPs (dATP, dGTP, dCTP, and dTTP) (TaKaRa)	0.4 mM
DNA polymerase TaKaRa Ex Taq (TaKaRa)	2.5 units
pCXND3-VB22B db	10 ng
Synthetic oligonucleotides, VB22B-fpvu, sc-rL15 or sc-fL15, and 33·115LR (primer D)	10 pmol

The reaction conditions were:

94°C (initial temperature) for 30 sec;

five cycles of 94°C for 15 sec and 72°C for 2 min;

five cycles of 94°C for 15 sec and 70°C for 2 min;

28 cycles of 94°C for 15 sec and 68°C for 2 min;

and final extension was at 72°C for 5 min.

[0160]

After the PCR products of about 400 bp were purified from agarose gel using the QIAquick Gel Extraction Kit (QIAGEN), the second-round PCR was carried out using aliquots of the respective PCR products according to the protocol described below.

The composition of the PCR reaction solution (50 μL in total) is shown below.

10x PCR Buffer (TaKaRa)	5 μL
dNTPs (dATP, dGTP, dCTP, and dTTP) (TaKaRa)	0.4 mM
DNA polymerase TaKaRa Ex Taq (TaKaRa)	2.5 units
First-round PCR product (two types)	1 μL
Synthetic oligonucleotide, 70·115HF and 33·115LR	10 pmol

The reaction conditions were:

94°C (initial temperature) for 30 sec;

five cycles of 94°C for 15 sec and 72°C for 2 min;

five cycles of 94°C for 15 sec and 70°C for 2 min;
28 cycles of 94°C for 15 sec and 68°C for 2 min;
and final extension was at 72°C for 5 min.

[0161]

The PCR products of about 800 bp were purified from agarose gel using the QIAquick Gel Extraction Kit (QIAGEN), and then digested with *Eco*RI and *Not*I (both from TaKaRa). The resulting DNA fragments were purified using the QIAquick PCR Purification Kit (QIAGEN), and then cloned into pBacPAK9 (Clontech) to construct pBacPAK9-scVB22B.

[0162]

A fragment to be inserted into the *Pvu*II site of pBacPAK9-scVB22B was prepared. Specifically, the fragment has a *Pvu*II recognition site at both ends and a nucleotide sequence, in which a gene encoding the VB22B-VH N-terminus is linked, *via* a $(Gly_4Ser)_3$ linker-encoding nucleotide sequence, to a gene encoding the amino acid sequence of an N-terminus-deleted VB22B-VH linked to VB22B-VL *via* the $(Gly_4Ser)_3$ linker. Two primers were newly designed to prepare the fragment by PCR. The forward primer for the fragment of interest, Fv2-f (primer E; SEQ ID NO: 176), was designed to have a *Pvu*II site at its 5' end and a VB22B-VH 5'-end sequence. The reverse primer for the fragment of interest, Fv2-r (primer F; SEQ ID NO: 177), was designed to hybridize to a DNA encoding the C terminus of VB22B-VL, and to have a *Pvu*II site, a nucleotide sequence encoding the $(Gly_4Ser)_3$ linker, and a nucleotide sequence hybridizing to a DNA encoding the N terminus of VB22B-VH. PCR was carried out using pBacPAK9-scVB22B as a template as described below.

[0163]

The composition of the PCR reaction solution (50 μL in total) is shown below.

10x PCR Buffer (TaKaRa)	5 μL
dNTPs (dATP, dGTP, dCTP, and dTTP) (TaKaRa)	0.4 mM
DNA polymerase TaKaRa Ex Taq (TaKaRa)	2.5 units
pBacPAK9-scVB22B	10 μg
Synthetic oligonucleotide, Fv2-f and Fv2-r	10 pmol

The reaction conditions were:
94°C (initial temperature) for 30 sec;
five cycles of 94°C for 15 sec and 72°C for 2 min;
five cycles of 94°C for 15 sec and 70°C for 2 min;
28 cycles of 94°C for 15 sec and 68°C for 2 min;
and final extension was at 72°C for 5 min.

[0164]

The PCR products of about 800 bp were purified from agarose gel using the QIAquick

Gel Extraction Kit (QIAGEN), and then cloned into the pGEM-T Easy Vector (Promega). After sequencing, the plasmid was digested with *Pvu*II (TaKaRa), and the fragment of interest was recovered. The recovered fragment was ligated to pBacPAK9-scVB22B pre-digested with *Pvu*II (TaKaRa) to construct pBacPAK9-VB22B sc$(Fv)_2$. After the resulting vector was digested with *Eco*RI and *Not*I (both from TaKaRa), the fragment of about 1,600 bp was purified from agarose gel using the QIAquick Gel Extraction Kit (QIAGEN). The fragment was then cloned into a pCXND3 expression vector to construct pCXND3-VB22B sc$(Fv)_2$.

[0165]

2.4 Expression of single-chain anti-human Mpl antibody in animal cells

A cell line stably expressing the single-chain antibody was prepared from CHO-DG44 cells as described below. Gene transfer was achieved by electroporation using a Gene Pulser II (Bio-Rad). An expression vector (25 μg) and 0.75 mL of CHO-DG44 cells suspended in PBS (1×10^7 cells/mL) were mixed. The resulting mixture was cooled on ice for 10 min, transferred into a cuvette, and pulsed at 1.5-kV and 25 μFD. After a ten-minute restoration period at room temperature, the electroporated cells were plated in CHO-S-SFMII medium (Invitrogen) containing 500 μg/mL Geneticin (Invitrogen). CHO cell lines expressing the single-chain antibody were established through selection. A cell line stably expressing VB22B sc$(Fv)_2$ and its culture supernatants were obtained by this method.

[0166]

The transient expression of the single-chain antibody was achieved using COS7 cells as described below. An expression vector (10 μg) and 0.75 mL of COS7 cells suspended in PBS (1×10^7 cells/mL) were mixed. The resulting mixture was cooled on ice for 10 min, transferred into a cuvette, and then pulsed at 1.5-kV and 25 μFD. After a ten-minute restoration period at room temperature, the electroporated cells were plated in DMEM/10% FBS medium (Invitrogen). The cells were incubated overnight and then washed with PBS. CHO-S-SFMII medium was added and the cells were cultured for about three days. The culture supernatants for preparing the VB22B diabody were thus prepared.

[0167]

2.5 Quantitation of single-chain anti-human Mpl antibodies in culture supernatants

The culture supernatant concentration of the single-chain anti-human Mpl antibody transiently expressed in COS cells was determined using surface plasmon resonance. A sensor chip CM5 (Biacore) was placed in Biacore 2000 (Biacore). ANTI-FLAG® M2 Monoclonal Antibody (Sigma-Aldrich) was immobilized onto the chip. An appropriate concentration of sample was injected over the chip surface at a flow rate of 5 mL/sec, and 50 mM diethylamine was used to dissociate the bound antibody. Changes in the mass during sample injection were recorded, and the sample concentration was calculated from the calibration curve prepared using

the mass changes of a standard sample. db12E10 (see WO 02/33073 and WO 02/33072) was used as the diabody standard, and 12E10 sc(Fv)$_2$ which has the same gene structure as that of sc(Fv)$_2$ was used as the sc(Fv)$_2$ standard.

[0168]

2.6 Purification of anti-human Mpl diabodies and single-chain antibodies

The culture supernatants of VB22B diabody-expressing COS7 cells or CHO cells was loaded onto an Anti-Flag M2 Affinity Gel (Sigma-Aldrich) column equilibrated with a 50 mM Tris-HCl buffer (pH7.4) containing 150 mM NaCl and 0.05% Tween20. The absorbed antibodies were eluted with 100 mM glycine-HCl (pH3.5). The fractions eluted were immediately neutralized with 1 M Tris-HCl (pH8.0), and loaded onto a HiLoad 26/60 Superdex 200 pg (Amersham Biosciences) column for gel filtration chromatography. PBS/0.01% Tween20 was used in the gel filtration chromatography.

[0169]

VB22B sc(Fv)$_2$ was purified from the culture supernatants of VB22B sc(Fv)$_2$-expressing COS7 cells or CHO cells under the same conditions used for purifying the diabodies. A large-scale preparation of VB22B sc(Fv)$_2$ was prepared by loading the CHO cell culture supernatants onto a Macro-Prep Ceramic Hydroxyapatite Type I (Bio-Rad) column equilibrated with a 20 mM phosphate buffer (pH6.8), and eluting the VB22B sc(Fv)$_2$ in a stepwise manner with 250 mM phosphate buffer (pH6.8). The eluted fraction was concentrated on an ultrafilter, and then fractionated by gel filtration chromatography using a HiLoad 26/60 Superdex 200 pg (Amersham Biosciences) column, and a fraction corresponding to the molecular weight range of about 40 kD to 70 kD was obtained. The fraction was loaded onto an Anti-Flag M2 Affinity Gel column equilibrated with a 50 mM Tris-HCl buffer (pH7.4) containing 150 mM NaCl and 0.05% Tween20. The absorbed antibody was eluted with 100 mM glycine-HCl (pH3.5). The eluted fraction was immediately neutralized with 1 M Tris-HCl (pH8.0), and loaded onto a HiLoad 26/60 Superdex 200 pg (Amersham Biosciences) column for gel filtration chromatography. 20 mM acetate buffer (pH6.0) containing 150 mM NaCl and 0.01% Tween80 was used in the gel filtration chromatography. In each purification step, the presence of the diabody and sc(Fv)$_2$ in the samples was confirmed by SDS-PAGE and Western blotting using an anti-Flag antibody (Sigma-Aldrich).

[0170]

2.7 Binding activity analyses of single-chain anti-human Mpl antibodies by flow cytometry

CHO-human Mpl, CHO-monkey Mpl, and CHO-mouse Mpl cells were recovered and suspended in FACS buffer (1% FBS/PBS) to a final concentration of 1 x 10^6 cells/mL. Cell suspensions were aliquoted at 100-μL/well into the Multiscreen-HV Filter Plates (Millipore). After centrifugation, the supernatant was removed. An appropriate concentration of diabody or

sc(Fv)2 was added into each well and incubated on ice for 30 min. The cells were washed once with 200 μL of FACS buffer, and incubated on ice for 30 min following the addition of 10 μg/mL ANTI-FLAG® M2 Monoclonal Antibody (Sigma-Aldrich). The cells were then washed once with 200 μL of FACS buffer, and a 100x-diluted FITC-labeled anti-mouse IgG antibody (Beckman Coulter) was added to the plate. The plate was incubated on ice for 30 min. After centrifugation, the supernatant was removed. The cells were suspended in 400 μL of FACS Buffer, and then analyzed by flow cytometry using EPICS ELITE ESP (Beckman Coulter). An analysis gate was set on the forward and side scatters of a histogram to include viable cell populations.

[0171]

The binding activity of the purified VB22B sc(Fv)$_2$ to various Mpl molecules expressed in CHO cells was determined (Fig. 2). VB22B sc(Fv)$_2$ was found to specifically bind to CHO-human Mpl and CHO-monkey Mpl but not to the host cell CHO or CHO-mouse Mpl. This binding characteristic of VB22B sc(Fv)$_2$ is comparable to those of VB22B IgG, indicating that the antibody binding site remains unaltered by reduction of molecular weight.

[0172]

2.8 Analyses of TPO-like agonistic activity for single-chain anti-human Mpl antibodies

TPO-like agonistic activity was assessed using BaF3-human Mpls or BaF3-monkey Mpls that proliferate in a TPO-dependent manner.

Cells from each cell line were washed twice with RPMI 1640/1% FBS (fetal bovine serum) (Invitrogen), and then suspended in RPMI 1640/10% FBS to a concentration of 4 x 10^5 cells/mL. Cell suspensions were aliquoted at 60-μL/well into a 96-well plate. Various concentrations of rhTPO (R&D) and COS7 culture supernatants or purified samples were prepared, and a 40-μL aliquot was added into each well. The plates were then incubated at 37°C under 5% CO_2 for 24 hr. Immediately after a 10-μL aliquot of WST-8 reagent (Cell Count Reagent SF; Nacalai Tesque) was added into each well, absorbance was measured at 450 nm (and at 655 nm as a control) using Benchmark Plus. After two hours of incubation, absorbance was again measured at 450 nm (and at 655 nm as a control). The WST-8 reagent changes colors at 450 nm in a color reaction that reflects the viable cell count. The TPO-like agonistic activity was assessed using the change in absorbance during the two-hour incubation as an index. EC_{50} values were computed using GraphPad Prism.

[0173]

TPO-like agonistic activity was assayed using the human leukemia cell line M-07e (purchased from DSMZ) which proliferates TPO-dependently. M-07e cells were washed twice with RPMI 1640/1% FBS, and then suspended in RPMI 1640/10% FBS to a concentration of 5 x 10^5 cells/mL. The resulting cell suspension was aliquoted at 50-μL/well into a 96-well plate.

Various concentrations of rhTPO and COS7 culture supernatants or purified samples were prepared, and a 50-μL aliquot was added into each well. The plates were then incubated at 37°C under 5% CO_2 for 48 hr. Immediately after a 10-μL aliquot of WST-8 reagent (Cell Count Reagent SF; Nacalai Tesque) was added to each well, absorbance of was measured at 450 nm (and at 655 nm as a control) using a Benchmark Plus. After four hours of incubation, absorbance was again measured at 450 nm (and at 655 nm as a control). The TPO-like agonistic activity was assayed using the change in absorbance during the four-hour incubation as an index.

[0174]

Purified VB22B IgG, VB22B diabody, and VB22B sc$(Fv)_2$ were assayed for their TPO-like agonistic activities using BaF3-human Mpl, BaF3-monkey Mpl, and M-07e. The results are shown in Figures 3, 4, and 5, respectively. The presence of bivalent antigen-binding domains in a single antibody molecule is essential for its agonistic activity. The distance and angle between two antigen-binding domains can also be important factors (see WO 02/33073 and WO 02/33072). Similar results were obtained for the newly isolated anti-human Mpl antibodies. Specifically, the agonistic activities of VB22B diabody and VB22B sc$(Fv)_2$ (EC_{50} = 61 pM and 27 pM in BaF-human Mpl, respectively) were higher than that of VB22B IgG (EC_{50} > 30 nM in BaF-human Mpl), and were equivalent to or higher than that of the naturally-occurring human TPO ligand (EC_{50} = 76 pM in BaF-human Mpl). The VB22B diabody activity was lower than that of VB22B sc$(Fv)_2$. This suggests that the structure of a single-chain antibody is greatly altered by its molecular shape and the length of the linker sequence, which in turn changes the agonistic activity. Sixteen types of the single-chain anti-human Mpl antibodies were obtained, each exhibiting a high agonistic activity. The amino acid sequences of the H chain and L chain variable regions of the representative antibodies are shown in Figures 6 and 7, respectively.

[0175]

2.9 Humanization of single-chain anti-human Mpl antibody

Antibody sequence data for the humanization of VB22B sc$(Fv)_2$ were obtained from the Kabat Database (ftp://ftp.ebi.ac.uk/pub/databases/kabat/), and homology searches were carried out independently for the H chain variable region and the L chain variable region. As a result, the H chain variable region was found to be highly homologous to DN13 (Smithson S. L. *et al.*, Mol Immunol. (1999) 36: 113-124). The L chain variable region was found to be highly homologous to ToP027 (Hougs L. *et al.*, J. Immunol. (1999) 162: 224-237). Humanized antibodies were prepared by inserting a complementarity-determining region (hereinafter abbreviated as "CDR") into the framework regions (hereinafter abbreviated as "FR") of the above antibodies. The humanized antibody sc$(Fv)_2$ was expressed in CHO-DG44 cells, and its

agonistic activity was assessed using BaF-human Mpl. The agonistic activity was used as an index to generate a humanized VB22B sc(Fv)$_2$ which has agonistic activity equivalent to that of murine VB22B sc(Fv)$_2$ by replacing one or more amino acids in its framework region.

[0176]

Specifically, synthetic oligo-DNAs of approximately 50 nucleotides in length were designed as to make 20 of these nucleotides available for hybridization, and the synthetic oligo-DNAs were assembled by PCR to prepare genes that encode the respective variable regions. Using the resulting genes, sc(Fv)$_2$ was similarly prepared by the method described in Example 2.3. The respective DNAs were cloned into a pCXND3expression vector to construct expression vectors pCXND3-hVB22B p-z sc(Fv)$_2$, pCXND3-hVB22B g-e sc(Fv)$_2$, pCXND3-hVB22B e sc(Fv)$_2$, pCXND3-hVB22B u2-wz4 sc(Fv)$_2$, and pCXND3-hVB22B q-wz5 sc(Fv)$_2$, to which the humanized VB22B sc(Fv)$_2$ is inserted. The nucleotide sequence and the amino acid sequence of hVB22B p-z sc(Fv)$_2$ in the plasmid are shown in SEQ ID NOs: 1 and 2. The nucleotide sequence and the amino acid sequence of hVB22B g-e sc(Fv)$_2$ are shown in SEQ ID NOs: 253 and 254. The nucleotide sequence and the amino acid sequence of hVB22B e sc(Fv)$_2$ are shown in SEQ ID NOs: 259 and 260. The nucleotide sequence and the amino acid sequence of hVB22B u2-wz4 sc(Fv)$_2$ are shown in SEQ ID NOs: 286 and 287. The nucleotide sequence and the amino acid sequence of hVB22B q-wz5 sc(Fv)$_2$ are shown in SEQ ID NOs: 292 and 293. The nucleotide sequence and the amino acid sequence of murine VB22B sc(Fv)$_2$ are shown in SEQ ID NOs: 263 and 264. The plasmids were expressed in CHO-DG44 cells and the culture supernatants were recovered by the method described in Example 2.4. Since the humanized VB22B sc(Fv)$_2$ does not contain a Flag tag, its purification from the culture supernatant was performed using a MG10-GST fusion protein. MG10 (Gln213 to Ala231) is one of the epitopes recognized by VB22B, as described in Example 1.8. The MG10-GST fusion protein was purified using Glutathione Sepharose 4B (Amersham Biosciences) according to the supplier's protocol. Then, the purified MG10-GST fusion protein was immobilized onto a HiTrap NHS-activated HP Column (Amersham Biosciences) to prepare an affinity column, according to the supplier's protocol. The culture supernatant of CHO cells expressing the humanized VB22B sc(Fv)$_2$ was loaded onto the MG10-GST fusion protein-immobilized column, which has been equilibrated with 50 mM Tris-HCl (pH7.4)/150 mM NaCl/0.01% Tween80. The adsorbed humanized VB22B sc(Fv)$_2$ was eluted with 100 mM glycine-HCl (pH3.5)/0.01% Tween80. Immediately after elution, the eluted fraction was neutralized with 1 M Tris-HCl (pH7.4), and was further subjected to gel filtration chromatography using a HiLoad 16/60 Superdex 200 pg (Amersham Biosciences). 20 mM citrate buffer (pH7.5) containing 300 mM NaCl and 0.01% Tween80 was used in the gel filtration chromatography. The TPO-like agonistic activities of the purified samples were similarly determined using the method described

in Example 2.8. The TPO-like agonistic activities of the purified murine VB22B sc(Fv)$_2$, hVB22B p-z sc(Fv)$_2$, hVB22B u2-wz4 sc(Fv)$_2$, hVB22B q-wz5 sc(Fv)$_2$, and humanized hVB22B e sc(Fv)$_2$ and hVB22B g-e sc(Fv)$_2$ in BaF3-human Mpl were assessed. The results are shown in Figures 19, 20, and 21. The humanized VB22B sc(Fv)$_2$ showed comparable agonistic activities, suggesting that the humanization has no influence on the activity. [0177]

[Example 3] Preparation of anti-Mpl diabodies by the AGS method

Anti-Mpl diabodies having agonistic activity were prepared by an Autocrine Growth Selection (AGS) method (see, WO 03/91424).

[0178]

3.1 Construction of a retrovirus library

Spleens were isolated from MRL/lpr mice immunized with shMPL-Flag by the method described in Example 1.5, and homogenized in TRIZOL Reagent (Invitrogen) using a Dounce homogenizer. After chloroform addition, the homogenized sample was shaken vigorously, the aqueous phase was removed and total RNA was extracted by isopropanol precipitation. mRNA was purified using a PolyATract System 1000 (Promega). Reverse transcription of 2.5 μg mRNA was carried out at 42°C for 50 min using the Superscript First strand synthesis system for RT-PCR (Invitrogen) and the included oligo-dT primers to prepare cDNA.

[0179]

The composition of the PCR reaction solution (250 μL) is shown below.

10x KOD Plus Buffer (Toyobo)	25 μL
2 mM dNTPs (dATP, dGTP, dCTP, and dTTP) (Toyobo)	25 μL
2.5 mM $MgSO_4$ (Toyobo)	10 μL
KOD Plus (Toyobo)	7.5 μL
Reverse transcription products	25 μL
Mixed primers complementary to H chain or L chain variable region	500 pmol

The reaction conditions were:

98°C (initial temperature) for 3 min;

32 cycles of 98°C for 20 sec, 58°C for 20 sec, and 72°C for 30 sec;

and final extension was at 72°C for 6 min.

[0180]

The H chain primer mix contained HS1 to HS19 (SEQ ID NOs: 178 to 196) and HA1 to HA4 (SEQ ID NOs: 197 to 200), which were mixed at the indicated ratios next to the sequence names in Table 1. The L chain primer mix contained LS1 to LS17 (SEQ ID NOs: 201 to 217), LSlambda (SEQ ID NO: 218), LA1 to LA5 (SEQ ID NOs: 219 to 222), and LAlambda (SEQ ID NO: 223). The respective PCR products were purified from agarose gel using the QIAquick Gel Extraction Kit (QIAGEN). The H chain and L chain variable regions were linked *via* the

$(Gly_4Ser)_1$ linker sequence by PCR using sc-S (SEQ ID NO: 224) and sc-AS (SEQ ID NO: 225) as described below.

[0181]

The composition of the PCR reaction solution (100 μL in total) is shown below.

10x KOD Plus Buffer (Toyobo)	10 μL
2 mM dNTPs (dATP, dGTP, dCTP, and dTTP) (Toyobo)	10 μL
2.5 mM $MgSO_4$ (Toyobo)	4 μL
KOD Plus (Toyobo)	2 μL
Fragment of H chain variable region	4 μL
Fragment of L chain variable region	4 μL

The first-round PCR conditions were:

94°C (initial temperature) for 3 min; and

seven cycles of 94°C for 1 min and 63°C for 4 min.

Then, sc-S and sc-AS (25 pmol each) were added to the first-round products.

The second-round PCR conditions were:

30 cycles of 94°C for 30 sec, 55°C for 2 min, and 72°C for 2 min;

and final extension was at 72°C for 6 min.

[0182]

The resulting product with an *Sfi*I restriction site at both ends was purified using the QIAquick PCR Purification Kit (QIAGEN), and incubated with the *Sfi*I restriction enzyme (TaKaRa) overnight at 50°C. The PCR product purified from agarose gel using the QIAquick Gel Extraction Kit (QIAGEN) was inserted into the *Sfi*I site of the viral vector pMX/IL3ssGFPHis.

[0183]

The resulting plasmid was constructed by inserting a GFP gene, which has an *Eco*RI site, mouse IL-3 signal sequence and *Sfi*I site at its 5' end; and an *Sfi*I site, His tag sequence, termination codon, and *Not*I site at its 3' end, between the *Eco*RI and *Not*I sites on the pMX viral vector (Onishi, M. *et al.*, Mol. Cell. Biol. 18: 3871-3879). The plasmid was introduced into the ElectroMAX DH10B T1 phage resistant cells (Invitrogen) by electroporation (settings: 2.5 kV, 25 μF, and 100Ω) using a Gene Pulser II (Bio-Rad). The cells were plated onto an LB-Agar plate containing 100 μg/mL ampicillin. After overnight incubation, 1×10^7 colonies were obtained. Colonies were recovered from the plate and plasmids were then extracted using the QIAGEN Plasmid Maxi Kit (QIAGEN).

[0184]

[Table 1]

SEQ ID NO:178	(HS1(4)) GCCCAGCCGGCCATGGCGGAKGTRMAGCTTCAGGAGTC
SEQ ID NO:179	(HS2(4)) GCCCAGCCGGCCATGGCGGAGGTBCAGCTBCAGCAGTC
SEQ ID NO:180	(HS3(3)) GCCCAGCCGGCCATGGCGCAGGTGCAGCTGAAGSASTC
SEQ ID NO:181	(HS4(4)) GCCCAGCCGGCCATGGCGGAGGTCCARCTGCAACARTC
SEQ ID NO:182	(HS5(7)) GCCCAGCCGGCCATGGCGCAGGTYCAGCTBCAGCARTC
SEQ ID NO:183	(HS6(2)) GCCCAGCCGGCCATGGCGCAGGTYCARCTGCAGCAGTC
SEQ ID NO:184	(HS7(1)) GCCCAGCCGGCCATGGCGCAGGTCCACGTGAAGCAGTC
SEQ ID NO:185	(HS8(2)) GCCCAGCCGGCCATGGCGGAGGTGAASSTGGTGGAATC
SEQ ID NO:186	(HS9(5)) GCCCAGCCGGCCATGGCGGAVGTGAWGYTGGTGGAGTC
SEQ ID NO:187	(HS10(2)) GCCCAGCCGGCCATGGCGGAGGTGCAGSKGGTGGAGTC
SEQ ID NO:188	(HS11(2)) GCCCAGCCGGCCATGGCGGAKGTGCAMCTGGTGGAGTC
SEQ ID NO:189	(HS12(2)) GCCCAGCCGGCCATGGCGGAGGTGAAGCTGATGGARTC
SEQ ID NO:190	(HS13(1)) GCCCAGCCGGCCATGGCGGAGGTGCARCTTGTTGAGTC
SEQ ID NO:191	(HS14(2)) GCCCAGCCGGCCATGGCGGARGTRAAGCTTCTCGAGTC
SEQ ID NO:192	(HS15(2)) GCCCAGCCGGCCATGGCGGAAGTGAARSTTGAGGAGTC
SEQ ID NO:193	(HS16(5)) GCCCAGCCGGCCATGGCGCAGGTTACTCTRAAAGWGTSTG
SEQ ID NO:194	(HS17(3.5)) GCCCAGCCGGCCATGGCGCAGGTCCAACTVCAGCARCC
SEQ ID NO:195	(HS18(0.7)) GCCCAGCCGGCCATGGCGGATGTGAACTTGGAAGTGTC
SEQ ID NO:196	(HS19(0.7)) GCCCAGCCGGCCATGGCGGAGGTGAAGGTCATCGAGTC
SEQ ID NO:197	(HA1(1)) GGAGCCGCCGCCGCCCGAGGAAACGGTGACCGTGGT
SEQ ID NO:198	(HA2(1)) GGAGCCGCCGCCGCCCGAGGAGACTGTGAGAGTGGT
SEQ ID NO:199	(HA3(1)) GGAGCCGCCGCCGCCCGCAGAGACAGTGACCAGAGT
SEQ ID NO:200	(HA4(1)) GGAGCCGCCGCCGCCCGAGGAGACGGTGACTGAGGT
SEQ ID NO:201	(LS1(1)) GGCGGCGGCGGCTCCGAYATCCAGCTGACTCAGCC
SEQ ID NO:202	(LS2(2)) GGCGGCGGCGGCTCCGAYATTGTTCTCWCCCAGTC
SEQ ID NO:203	(LS3(5)) GGCGGCGGCGGCTCCGAYATTGTGMTMACTCAGTC
SEQ ID NO:204	(LS4(3.5)) GGCGGCGGCGGCTCCGAYATTGTGYTRACACAGTC
SEQ ID NO:205	(LS5(4)) GGCGGCGGCGGCTCCGAYATTGTRATGACMCAGTC
SEQ ID NO:206	(LS6(7)) GGCGGCGGCGGCTCCGAYATTMAGATRAMCCAGTC
SEQ ID NO:207	(LS7(6)) GGCGGCGGCGGCTCCGAYATTCAGATGAYDCAGTC
SEQ ID NO:208	(LS8(1.5)) GGCGGCGGCGGCTCCGAYATYCAGATGACACAGAC
SEQ ID NO:209	(LS9(2)) GGCGGCGGCGGCTCCGAYATTGTTCTCAWCCAGTC
SEQ ID NO:210	(LS10(3.5)) GGCGGCGGCGGCTCCGAYATTGWGCTSACCCAATC
SEQ ID NO:211	(LS11(8)) GGCGGCGGCGGCTCCGAYATTSTRATGACCCARTC
SEQ ID NO:212	(LS12(8)) GGCGGCGGCGGCTCCGAYRTTKTGATGACCCARAC
SEQ ID NO:213	(LS13(6)) GGCGGCGGCGGCTCCGAYATTGTGATGACBCAGKC
SEQ ID NO:214	(LS14(2)) GGCGGCGGCGGCTCCGAYATTGTGATAACYCAGGA
SEQ ID NO:215	(LS15(2)) GGCGGCGGCGGCTCCGAYATTGTGATGACCCAGWT
SEQ ID NO:216	(LS16(1)) GGCGGCGGCGGCTCCGAYATTGTGATGACACAACC
SEQ ID NO:217	(LS17(1)) GGCGGCGGCGGCTCCGAYATTTTGCTGACTCAGTC
SEQ ID NO:218	(LSlambda(1)) GGCGGCGGCGGCTCCGATGCTGTTGTGACTCAGGAATC
SEQ ID NO:219	(LA1(4)) GGAATTCGGCCCCCGAGGCCTTGATTTCCAGCTTGG
SEQ ID NO:220	(LA2(4)) GGAATTCGGCCCCCGAGGCCTTTATTTCCAGCTTGG
SEQ ID NO:221	(LA4(4)) GGAATTCGGCCCCCGAGGCCTTTATTTCCAACTTTG
SEQ ID NO:222	(LA5(4)) GGAATTCGGCCCCCGAGGCCTTCAGCTCCAGCTTGG
SEQ ID NO:223	(LAlambda(1)) GGAATTCGGCCCCCGAGGCCCCTAGGACAGTCAGTTTGG

[0185]

3.2 Establishment of autonomously replicating cell lines by the AGS method

The resulting library was transfected into a packaging cell, Pt-E, (Morita, S. *et al.*, Gene therapy 7: 1063-1066) using FuGENE 6 (Roche Diagnostics). Specifically, Pt-E was plated onto 6-cm dishes and cultured in DMEM/10% FBS (Invitrogen). A mixture of FuGENE 6 and the library was added to the plate the following day. The culture medium was exchanged the next day, and the culture supernatant was collected 24 hours after that. 10 μg/mL polybrene (Hexadimethrine Bromide; Sigma) and 2 ng/mL mIL-3 were added to the culture supernatant containing recombinant virus particles. The viral solution was used to infect the BaF-monkey Mpl target cells. The cells were washed with PBS the following day, and suspended in RPMI 1640/10% FBS without mIL-3. The suspension was plated onto a 96-well plate at a cell density of 1,000 cells/well. Autonomously replicating cell lines (AB317 and AB324) were obtained after seven days of incubation. Genomic DNAs were extracted from these cells using a DNeasy Tissue Kit (QIAGEN), and the antibody genes were amplified by PCR.

[0186]

The composition of the PCR reaction solution (50 μL in total) is shown below.

10x LA Taq Buffer (TaKaRa)	5 μL
2 mM dNTPs (dATP, dGTP, dCTP, and dTTP) (TaKaRa)	5 μL
2.5 mM $MgCl_4$ (TaKaRa)	5 μL
TaKaRa LA Taq (TaKaRa)	0.5 μL
Genomic DNA	0.5 μg
AGSdbS1 (SEQ ID NO: 226) and AGSdbA1 (SEQ ID NO: 227)	25 pmol

The reaction conditions were:

94°C (initial temperature) for 1 min;

30 cycles of 94°C for 30 sec, 60°C for 30 sec, and 70°C for 1 min; and final extension was at 72°C for 6 min.

[0187]

The nucleotide sequence and the amino acid sequence of the H chain of cloned AB317 are shown in SEQ ID NOs: 154 and 155. The nucleotide sequence and the amino acid sequence of AB317 L chain are shown in SEQ ID NOs: 156 and 157. The nucleotide sequence and the amino acid sequence of AB324 H chain are shown in SEQ ID NOs: 158 and 159. The nucleotide sequence and the amino acid sequence of AB324 L chain are shown in SEQ ID NOs: 160 and 161. [0188]

3.3 Activity assays of the diabodies obtained by AGS method

Each of the anti-Mpl diabodies obtained above was inserted into the pCXND3 expression vector. The PCR primers used are a synthetic oligonucleotide complementary to the

5' end of the diabody and containing an *Eco*RI site, and a synthetic oligonucleotide complementary to the nucleotide sequence of the 3' end of the diabody and containing a FLAG tag and a *Not*I site. The PCR product thus obtained was inserted into pCXND3 between the *Eco*RI and *Not*I sites. The diabody was expressed transiently in COS7 cells by the method described in Example 2.4. The culture supernatant was removed and the activity of the diabody was evaluated.

The binding activities of the diabodies were assessed by flow cytometry using CHO cells that express Mpl derived from various species (Fig. 8). AB317 was proven to bind to CHO-mouse Mpl.

[0189]

The TPO-like agonistic activities of the diabodies were evaluated using BaF-human Mpl, BaF-monkey Mpl, and BaF-mouse Mpl (Figures 9, 10, and 11). AB317 had the highest agonistic activity against human, monkey, and mouse Mpl, whereas AB324 showed the highest agonistic activity against human and monkey Mpl.

This proves that anti-Mpl diabodies having high agonistic activity can be obtained by the AGS method.

[0190]

[Example 4] Agonistic activity assays of the anti-Mpl antibodies against mutant Mpl in congenital amegakaryocytic thrombocytopenia (CAMT) patients

4.1 Establishment of BaF3 cell lines introduced with the mutant Mpl observed in CAMT patients

Mutations on G305C (R102P), C769T (R257C), and C823A (P275T) have been reported in the Mpl gene of CAMT patients. The respective expression vectors carrying the Mpl gene mutations were constructed and introduced into BaF3 cells. The following Mpl gene fragments were constructed: normal Mpl gene (nucleotide sequence, SEQ ID NO: 246; amino acid sequence, SEQ ID NO: 123); gene G305C in which C is substituted for 305th nucleotide G relative to the initiation codon (nucleotide sequence, SEQ ID NO: 247; amino acid sequence, SEQ ID NO: 248); gene C769T in which T is substituted for 769th nucleotide C (nucleotide sequence, SEQ ID NO: 249; amino acid sequence, SEQ ID NO: 250); and gene C823A in which A is substituted for 823rd nucleotide C (nucleotide sequence, SEQ ID NO: 251; amino acid sequence, SEQ ID NO: 252). The above-described DNA fragments were digested with *Eco*RI and *Sal*I, and inserted between the *Eco*RI and *Sal*I sites on the animal cell expression vector pCOS2-Ha to prepare pCOS2-hMPLfullG305C, pCOS2-hMPLfullC769T, and pCOS2-hMPLfullC823A.

[0191]

The genes were introduced into BaF3 cells by the procedure described in Example 1.1.1 to establish BaF3 cell lines expressing each Mpl gene: BaF3-human MPL (G305C),

BaF3-human MPL (C769T), and BaF3-human MPL (C823A). After the selection, the cells were cultured and passaged using RPMI 1640 containing 1 ng/mL mIL-3 and 10% FBS.

[0192]

4.2 Preparation of anti-human Mpl diabody and sc(Fv)$_2$

Among the amino acid sequences shown in Figures 6 and 7, expression vectors were prepared for the diabodies VB8B, VB45B, VB33, VB140, VB157, and TA136 using the same procedure described in Example 2.2.2. The prepared expression vectors were introduced into COS7 cells by the same procedure described in Example 2.2.4. The supernatant concentration of each diabody was determined by the method of Example 2.2.5. The sc(Fv)$_2$ expression vector for TA136 was prepared by the same procedure described in Example 2.2.3. The vector was introduced into CHO-DG44 cells by the same procedure described in Example 2.2.4. sc(Fv)$_2$ was purified from the culture supernatant thus obtained using the same method described in Example 2.2.6.

[0193]

4.3 Agonistic activity assays of sc(Fv)$_2$ and the anti-human Mpl diabodies

The prepared diabodies and sc(Fv)$_2$ were assayed for their agonistic activities in normal Mpl and mutant Mpl in BaF3 cells by the same procedure described in Example 2.2.8. The agonistic activities in BaF3-human Mpl and BaF3-human Mpl (G305C) were compared using the culture supernatants of cells expressing the diabodies. The TA136 diabody (TA136 db) was shown to have a low agonistic activity in BaF3-human Mpl cells expressing the normal Mpl gene, and a high agonistic activity in BaF3-human Mpl (G305C) cells expressing the mutant Mpl gene. hTPO and the rest of the diabodies did not show a high agonistic activity in BaF3-human Mpl (G305C) cells (Figures 12 and 13).

[0194]

In addition, the agonistic activities of the TA136 diabody and TA136 sc(Fv)$_2$ in BaF3-human Mpl, BaF3-human Mpl (G305C), BaF3-human Mpl (C769T), and BaF3-human Mpl (C823A) cells were assessed using a purified sample of the diabody. Compared with hTPO and the TA136 diabody, TA136 sc(Fv)$_2$ exhibited a higher agonistic activity in all three types of the TPO receptor mutant cell lines (Figures 15, 16 and 17). Furthermore, it was shown that in BaF3-human Mpl cells expressing the normal Mpl gene, the TA136 diabody exhibited a lower activity than hTPO. However, an agonistic activity equivalent to that of hTPO was achieved by converting the diabody into sc(Fv)$_2$ (Fig. 14).

[Brief Description of the Drawings]

[0195]

[Fig. 1] Fig. 1 demonstrates the strategy for preparing single-chain antibody sc(Fv)$_2$.

[Fig. 2] Fig. 2 illustrates the assessment of VB22B sc(Fv)$_2$ binding activity using an

Mpl-expressing CHO cell line. Purified VB22B sc(Fv)$_2$ was used.

[Fig. 3] Fig. 3 illustrates the assessment of VB22B antibody agonistic activity using BaF-human Mpl.

[Fig. 4] Fig. 4 illustrates the assessment of VB22B antibody agonistic activity using BaF-monkey Mpl.

[Fig. 5] Fig. 5 illustrates the assessment of VB22B antibody agonistic activity using M-07e.

[Fig. 6] Fig. 6 shows the amino acid sequences of anti-human Mpl antibodies (H chains) that exhibit higher agonistic activities when converted into minibodies.

[Fig. 7] Fig. 7 shows the amino acid sequences of anti-human Mpl antibodies (L chains) which exhibit higher agonistic activities when converted into minibodies.

[Fig. 8] Fig. 8 illustrates the binding activity assessment of AB317 diabody using Mpl-expressing CHO cells. Both VB22B diabody (solid line) and AB317 diabody (broken line) were obtained from COS7 culture supernatants.

[Fig. 9] Fig. 9 illustrates the agnostic activity assessment of AB324 and AB317 diabodies using BaF-human Mpl.

[Fig. 10] Fig. 10 illustrates the agnostic activity assessment of AB324 and AB317 diabodies using BaF-monkey Mpl.

[Fig. 11] Fig. 11 illustrates the agnostic activity assessment of AB324 and AB317 diabodies using BaF-mouse Mpl.

[Fig. 12] Fig. 12 shows the agonistic activities of diabodies and hTPO in BaF3-human Mpl cells. The Y-axis shows OD at 450/655 nm, and the X-axis represents concentration.

[Fig. 13] Fig. 13 shows the agonistic activities of diabodies and hTPO in BaF3-human Mpl (G305C) cells. The Y-axis shows OD at 450/655 nm, and the X-axis represents concentration.

[Fig. 14] Fig. 14 shows the agonistic activities of TA136 db and TA136 sc(Fv)$_2$ in BaF3-human Mpl cells. The Y-axis shows OD at 450/655 nm and the X-axis represents concentration.

[Fig. 15] Fig. 15 shows the agonistic activities of TA136 db and TA136 sc(Fv)$_2$ in BaF3-human Mpl (G305C) cells. The Y-axis shows OD at 450/655 nm, and the X-axis represents concentration.

[Fig. 16] Fig. 16 shows the agonistic activities of TA136 db and TA136 sc(Fv)$_2$ in BaF3-human Mpl (C769T) cells. The Y-axis shows OD at 450/655 nm, and the X-axis represents concentration.

[Fig. 17] Fig. 17 shows the agonistic activities of TA136 db and TA136 sc(Fv)$_2$ in BaF3-human Mpl (C823A) cells. The Y-axis shows OD at 450/655 nm, and the X-axis

represents concentration.

[Fig. 18] Fig. 18 shows the positions of FRs and CDRs in humanized heavy chain sequences (hVB22B p-z, hVB22B g-e, hVB22B e, hVB22B u2-wz4, and hVB22B q-wz5:VH), and humanized light chain sequences (hVB22B p-z, hVB22B g-e, hVB22B e, hVB22B u2-wz4, and hVB22B q-wz5:VL).

[Fig. 19] Fig. 19 shows the TPO-like agonistic activities of murine VB22B sc$(Fv)_2$, hVB22B e sc$(Fv)_2$, and hVB22B g-e sc$(Fv)_2$ in BaF3-human Mpl. The Y-axis shows absorbance ratio (450nm/655nm), and the X-axis represents concentration.

[Fig. 20] Fig. 20 shows the TPO-like agonistic activities of murine VB22B sc$(Fv)_2$, hVB22B p-z sc$(Fv)_2$, and hVB22B u2-wz4 sc$(Fv)_2$ in BaF3-human Mpl. The Y-axis shows absorbance ratio (450nm/655nm), and the X-axis represents concentration.

[Fig. 21] Fig. 21 shows the TPO-like agonistic activities of murine VB22B sc$(Fv)_2$ and hVB22B q-wz5 sc$(Fv)_2$ in BaF3-human Mpl. The Y-axis shows absorbance ratio (450nm/655nm), and the X-axis represents concentration.

[Sequence Listing]

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Arg Trp Phe Leu Asp Cys
1 5

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<400> 54

Ser Ser Trp Met Asn
1 5

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Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys
1 5 10 15

Gly

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<400> 56

Ala Arg Lys Thr Ser Trp Phe Ala Tyr
1 5

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Ser Asp Tyr Ala Trp Ser
1 5

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Tyr Ile Thr Tyr Ser Gly Tyr Ser Ile Tyr Asn Pro Ser Leu Lys Ser
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Gly Tyr Asp Asn Met Asp Tyr
1 5

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Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
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Arg Met Ser Asn Leu Ala Ser
1 5

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<400> 62

Met Gln His Leu Glu Tyr Pro Phe Thr
1 5

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<400> 63

Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
1 5 10 15

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Arg Met Ser Asn Leu Ala Ser
1 5

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Met Gln His Leu Glu Tyr Pro Tyr Thr
1 5

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Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
1 5 10 15

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<400> 67

Arg Met Ser Asn Leu Ala Ser
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Met Gln His Leu Glu Tyr Pro Tyr Thr
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Met Gln His Leu Glu Tyr Pro Tyr Thr
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Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
1 5 10 15

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Arg Met Ser Asn Leu Ala Ser
1 5

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Met Gln His Leu Glu Tyr Pro Tyr Thr

1 5

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Arg Met Ser Asn Leu Ala Ser
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Met Gln His Leu Glu Tyr Pro Tyr Thr
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Arg Met Ser Asn Leu Ala Ser
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Met Gln His Leu Glu Tyr Pro Tyr Thr
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Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
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Met Gln His Leu Glu Tyr Pro Tyr Thr
1 5

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Met Gln His Val Glu Tyr Pro Tyr Thr
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Arg Met Ser Asn Leu Ala Ser
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Met Gln His Leu Glu Tyr Pro Tyr Thr
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Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
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Arg Met Ser Asn Leu Ala Ser
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Met Gln His Leu Glu Tyr Pro Tyr Thr
1 5

<210> 93
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<400> 93

Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
1 5 10 15

<210> 94
<211> 7
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<213> Mus musculus

<400> 94

Arg Met Ser Asn Leu Ala Ser
1 5

<210> 95
<211> 9
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<400> 95

Met Gln His Ile Glu Tyr Pro Phe Thr
1 5

<210> 96
<211> 16
<212> PRT
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Arg Ser Ser Lys Ser Leu Leu Tyr Ser Asn Gly Asn Thr Tyr Leu Tyr
1 5 10 15

<210> 97
<211> 7
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<213> Mus musculus

<400> 97

Arg Met Ser Asn Leu Ala Ser
1 5

<210> 98
<211> 9
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<400> 98

Met Gln His Leu Glu Tyr Pro Tyr Thr
1 5

<210> 99
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Arg Ser Ser Lys Ser Leu Leu Tyr Ser Asn Gly Asn Ile Tyr Leu Tyr
1 5 10 15

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Arg Met Ser Asn Leu Ala Ser
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Met Gln His Leu Glu Tyr Pro Tyr Thr
1 5

<210> 102
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<400> 102

Arg Ser Ser Lys Ser Leu Leu His Asn Asn Gly Asn Thr Tyr Leu Tyr
1 5 10 15

<210> 103
<211> 7
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<213> Mus musculus

<400> 103

Arg Met Ser Asn Leu Ala Ser
1 5

<210> 104
<211> 9

<212> PRT
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<400> 104

Met Gln His Ile Glu Tyr Pro Phe Thr
1 5

<210> 105
<211> 16
<212> PRT
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<400> 105

Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
1 5 10 15

<210> 106
<211> 7
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<400> 106

Arg Met Ser Asn Leu Ala Ser
1 5

<210> 107
<211> 9
<212> PRT
<213> Mus musculus

<400> 107

Met Gln His Leu Glu Tyr Pro Tyr Thr
1 5

<210> 108
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<213> Mus musculus

<400> 108

Arg Ala Ser Glu Ser Val Glu Tyr Tyr Gly Thr Ser Leu Met Gln
1 5 10 15

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<211> 7
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Gly Ala Ser Asn Val Glu Ser
1 5

<210> 110
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<400> 110

Gln Gln Ser Arg Lys Val Pro Trp Thr

1 5

<210> 111
<211> 11
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<400> 111

Lys Ala Ser Gln Asn Val Gly Asn Ile Ile Ala
1 5 10

<210> 112
<211> 7
<212> PRT
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<400> 112

Leu Ala Ser Tyr Arg Tyr Ser
1 5

<210> 113
<211> 9
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<400> 113

Gln Gln Tyr Ser Ser Ser Pro Leu Thr
1 5

<210> 114
<211> 12
<212> PRT
<213> Mus musculus

<400> 114

Ser Ala Ser Ser Ser Val Ser Ser Ser His Leu Tyr
1 5 10

<210> 115
<211> 7
<212> PRT
<213> Mus musculus

<400> 115

Ser Thr Ser Asn Leu Ala Ser
1 5

<210> 116
<211> 9
<212> PRT
<213> Mus musculus

<400> 116

His Gln Trp Ser Ser Tyr Pro Trp Thr
1 5

<210> 117
<211> 354
<212> DNA

<213> Mus musculus

<400> 117

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cctggaaagg gtcttgagtg gattggacgg atttatcctg gagatggaga aactatctac 180

aatgggaaat tcagggtcaa ggccacactg actgcagaca aatcctccag cacagcctac 240

atggatatca gcagcctgac atctgaggac tctgcggtct acttctgtgc aagaggctat 300

gatgattact cgtttgctta ctggggccaa gggactctgg tcactgtctc tgca 354

<210> 118
<211> 118
<212> PRT
<213> Mus musculus

<400> 118

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Thr Asn Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn Gly Lys Phe

50 55 60

Arg Val Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Asp Ile Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 119
<211> 336
<212> DNA
<213> Mus musculus

<400> 119
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ttcctgcaga ggccaggcca gtctcctcaa ctcctgatat atcggatgtc caaccttgcc 180

tcaggagtcc cagataggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc 240

agtagagtgg aggctgagga tgtgggtgtt tattactgta tgcaacatat agaatatcct 300

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<210> 120
<211> 112
<212> PRT
<213> Mus musculus

<400> 120

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Ile Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Ile Glu Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 121
<211> 762
<212> DNA
<213> Mus musculus

<400> 121
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ggttcggata ttgtgatgac tcaggctgca ccctctatac ctgtcactcc tggagagtca 480

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cttgcctcag gagtcccaga taggttcagt ggcagtgggt caggaactgc tttcacactg 660

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tatcctttta cgttcggatc ggggaccaag ctggaaataa aa 762

<210> 122
<211> 254

<212> PRT
<213> Mus musculus

<400> 122

Met Glu Trp Pro Leu Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
1 5 10 15

Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
20 25 30

Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe
35 40 45

Thr Asn Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu
50 55 60

Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn
65 70 75 80

Gly Lys Phe Arg Val Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser
85 90 95

Thr Ala Tyr Met Asp Ile Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
100 105 110

Tyr Phe Cys Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly
115 120 125

Gln Gly Thr Leu Val Thr Val Ser Ala Gly Gly Gly Gly Ser Asp Ile
130 135 140

Val Met Thr Gln Ala Ala Pro Ser Ile Pro Val Thr Pro Gly Glu Ser
145 150 155 160

Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly
165 170 175

Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser Pro Gln
180 185 190

Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro Asp Arg
195 200 205

Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile Ser Arg
210 215 220

Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His Ile Glu
225 230 235 240

Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
245 250

<210> 123
<211> 635
<212> PRT

<213> Homo sapiens

<400> 123

Met Pro Ser Trp Ala Leu Phe Met Val Thr Ser Cys Leu Leu Leu Ala
1 5 10 15

Pro Gln Asn Leu Ala Gln Val Ser Ser Gln Asp Val Ser Leu Leu Ala
20 25 30

Ser Asp Ser Glu Pro Leu Lys Cys Phe Ser Arg Thr Phe Glu Asp Leu
35 40 45

Thr Cys Phe Trp Asp Glu Glu Glu Ala Ala Pro Ser Gly Thr Tyr Gln
50 55 60

Leu Leu Tyr Ala Tyr Pro Arg Glu Lys Pro Arg Ala Cys Pro Leu Ser
65 70 75 80

Ser Gln Ser Met Pro His Phe Gly Thr Arg Tyr Val Cys Gln Phe Pro
85 90 95

Asp Gln Glu Glu Val Arg Leu Phe Phe Pro Leu His Leu Trp Val Lys
100 105 110

Asn Val Phe Leu Asn Gln Thr Arg Thr Gln Arg Val Leu Phe Val Asp
115 120 125

Ser Val Gly Leu Pro Ala Pro Pro Ser Ile Ile Lys Ala Met Gly Gly
130 135 140

Ser Gln Pro Gly Glu Leu Gln Ile Ser Trp Glu Glu Pro Ala Pro Glu
145 150 155 160

Ile Ser Asp Phe Leu Arg Tyr Glu Leu Arg Tyr Gly Pro Arg Asp Pro
165 170 175

Lys Asn Ser Thr Gly Pro Thr Val Ile Gln Leu Ile Ala Thr Glu Thr
180 185 190

Cys Cys Pro Ala Leu Gln Arg Pro His Ser Ala Ser Ala Leu Asp Gln
195 200 205

Ser Pro Cys Ala Gln Pro Thr Met Pro Trp Gln Asp Gly Pro Lys Gln
210 215 220

Thr Ser Pro Ser Arg Glu Ala Ser Ala Leu Thr Ala Glu Gly Gly Ser
225 230 235 240

Cys Leu Ile Ser Gly Leu Gln Pro Gly Asn Ser Tyr Trp Leu Gln Leu
245 250 255

Arg Ser Glu Pro Asp Gly Ile Ser Leu Gly Gly Ser Trp Gly Ser Trp
260 265 270

Ser Leu Pro Val Thr Val Asp Leu Pro Gly Asp Ala Val Ala Leu Gly
275 280 285

Leu Gln Cys Phe Thr Leu Asp Leu Lys Asn Val Thr Cys Gln Trp Gln
290 295 300

Gln Gln Asp His Ala Ser Ser Gln Gly Phe Phe Tyr His Ser Arg Ala
305 310 315 320

Arg Cys Cys Pro Arg Asp Arg Tyr Pro Ile Trp Glu Asn Cys Glu Glu
325 330 335

Glu Glu Lys Thr Asn Pro Gly Leu Gln Thr Pro Gln Phe Ser Arg Cys
340 345 350

His Phe Lys Ser Arg Asn Asp Ser Ile Ile His Ile Leu Val Glu Val
355 360 365

Thr Thr Ala Pro Gly Thr Val His Ser Tyr Leu Gly Ser Pro Phe Trp
370 375 380

Ile His Gln Ala Val Arg Leu Pro Thr Pro Asn Leu His Trp Arg Glu
385 390 395 400

Ile Ser Ser Gly His Leu Glu Leu Glu Trp Gln His Pro Ser Ser Trp
405 410 415

Ala Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly Glu Gly His
420 425 430

Gln Asp Trp Lys Val Leu Glu Pro Pro Leu Gly Ala Arg Gly Gly Thr
435 440 445

Leu Glu Leu Arg Pro Arg Ser Arg Tyr Arg Leu Gln Leu Arg Ala Arg
450 455 460

Leu Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ser Trp Ser Asp Pro
465 470 475 480

Thr Arg Val Glu Thr Ala Thr Glu Thr Ala Trp Ile Ser Leu Val Thr
485 490 495

Ala Leu His Leu Val Leu Gly Leu Ser Ala Val Leu Gly Leu Leu Leu
500 505 510

Leu Arg Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu
515 520 525

Trp Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg
530 535 540

Asp Thr Ala Ala Leu Ser Pro Pro Lys Ala Thr Val Ser Asp Thr Cys
545 550 555 560

Glu Glu Val Glu Pro Ser Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu
565 570 575

Arg Thr Pro Leu Pro Leu Cys Ser Ser Gln Ala Gln Met Asp Tyr Arg
580 585 590

Arg Leu Gln Pro Ser Cys Leu Gly Thr Met Pro Leu Ser Val Cys Pro
595 600 605

Pro Met Ala Glu Ser Gly Ser Cys Cys Thr Thr His Ile Ala Asn His
610 615 620

Ser Tyr Leu Pro Leu Ser Tyr Trp Gln Gln Pro
625 630 635

<210> 124
<211> 122
<212> PRT
<213> Mus musculus

<400> 124

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile

35 40 45

Gly Arg Thr Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Trp Ile Leu Ala Asp Gly Gly Tyr Ser Phe Ala Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ala
115 120

<210> 125
<211> 112
<212> PRT
<213> Mus musculus

<400> 125

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Ile Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Ile Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Phe Thr Phe Gly Thr Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 126
<211> 118
<212> PRT
<213> Mus musculus

<400> 126

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Ile Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Tyr Ala Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 127
<211> 112
<212> PRT
<213> Mus musculus

<400> 127

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 128
<211> 118
<212> PRT
<213> Mus musculus

<400> 128

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser

20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Asn Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Phe Gly Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 129
<211> 112
<212> PRT
<213> Mus musculus

<400> 129

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Ala Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Thr Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 130
<211> 118
<212> PRT
<213> Mus musculus

<400> 130

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ser Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Ser Gly Tyr Ala Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 131
<211> 112
<212> PRT
<213> Mus musculus

<400> 131

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 132
<211> 118
<212> PRT
<213> Mus musculus

<400> 132

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala

1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Arg Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Ser Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 133
<211> 112
<212> PRT
<213> Mus musculus

<400> 133

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 134
<211> 118
<212> PRT
<213> Mus musculus

<400> 134

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Arg Ala Phe Gly Tyr Ala Phe Ser Asn Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Asn Asn Asn Gly Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Tyr Gly Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 135
<211> 112
<212> PRT

<213> Mus musculus

<400> 135

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Ala Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 136
<211> 115
<212> PRT
<213> Mus musculus

<400> 136

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Trp Val Asn Trp Val Lys Gln Arg Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile His Pro Ser Asp Ser Glu Thr His Cys Asn Gln Lys Phe
50 55 60

Lys Arg Lys Ala Thr Leu Thr Val Asn Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Ile Gln Leu His Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Thr Ser Gly Gly Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ala
115

<210> 137

<211> 112
<212> PRT
<213> Mus musculus

<400> 137

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu Tyr Ser
20 25 30

Asn Gly Asn Ile Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 138
<211> 118

<212> PRT
<213> Mus musculus

<400> 138

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Asn Asn Asn Gly Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Tyr Gly Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 139
<211> 112
<212> PRT
<213> Mus musculus

<400> 139

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Ala Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 140
<211> 118
<212> PRT
<213> Mus musculus

<400> 140

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Thr Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Ala Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Ser Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Tyr Gly Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala

115

<210> 141
<211> 112
<212> PRT
<213> Mus musculus

<400> 141

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Met Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Val Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 142
<211> 118
<212> PRT
<213> Mus musculus

<400> 142

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Pro Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Val Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Tyr Gly Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 143
<211> 112
<212> PRT
<213> Mus musculus

<400> 143

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 144
<211> 118
<212> PRT
<213> Mus musculus

<400> 144

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Leu Asn Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Arg Ser
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr
65 70 75 80

Met Gln Phe Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Asp Gly Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr

100 105 110

Leu Val Thr Val Ser Ala
115

<210> 145
<211> 112
<212> PRT
<213> Mus musculus

<400> 145

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 146
<211> 115
<212> PRT
<213> Mus musculus

<400> 146

Gln Val Gln Leu Gln Gln Pro Gly Thr Glu Leu Val Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Trp Val Asn Trp Val Lys Gln Arg Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile His Pro Tyr Asp Ser Glu Thr His Tyr Asn Gln Lys Phe
50 55 60

Lys Asn Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Ile Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Gly Gly Trp Phe Ala Ser Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ala
115

<210> 147
<211> 112
<212> PRT
<213> Mus musculus

<400> 147

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu Tyr Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Thr Ile
65 70 75 80

Ser Ser Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 148
<211> 115
<212> PRT
<213> Mus musculus

<400> 148

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile His Pro Phe Asp Ser Glu Thr His Cys Ser Gln Lys Phe
50 55 60

Lys Asn Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr
65 70 75 80

Ile Gln Phe Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys

85 90 95

Ser Ser Gly Gly Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ala
115

<210> 149
<211> 112
<212> PRT
<213> Mus musculus

<400> 149

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Ser Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu Tyr Ser
20 25 30

Asn Gly Asn Ile Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 150
<211> 118
<212> PRT
<213> Mus musculus

<400> 150

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Thr Asn Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn Gly Lys Phe
50 55 60

Arg Val Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Ile Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 151
<211> 112
<212> PRT
<213> Mus musculus

<400> 151

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Asn
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Ile Glu Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 152
<211> 118
<212> PRT
<213> Mus musculus

<400> 152

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Asn Ser
20 25 30

Trp Met Asn Trp Val Asn Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Ile Tyr Asn Gly Asn Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Ile Ala Tyr

65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Thr Ser Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala
115

<210> 153
<211> 112
<212> PRT
<213> Mus musculus

<400> 153

Asp Ile Val Met Thr Gln Ala Ala Pro Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Leu Glu Tyr Pro Tyr Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 154
<211> 423
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(423)
<223>

<400> 154
atg gtt ctt gcc agc tct acc acc agc atc cac acc atg ctg ctc ctg 48
Met Val Leu Ala Ser Ser Thr Thr Ser Ile His Thr Met Leu Leu Leu
1 5 10 15

ctc ctg atg ctg gcc cag ccg gcc atg gcg gaa gtg aag ctg gtg gag 96
Leu Leu Met Leu Ala Gln Pro Ala Met Ala Glu Val Lys Leu Val Glu
20 25 30

tct ggg gga ggc tta gtg aag cct gga ggg tcc cgg aaa ctc tcc tgt 144
Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Arg Lys Leu Ser Cys
35 40 45

gca gcc tct gga ttc act ttc agt agc tat acc atg tct tgg gtt cgc 192
Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Thr Met Ser Trp Val Arg
50 55 60

cag act ccg gcg aag agg ctg gag tgg gtc gca acc att agt agt ggc 240
Gln Thr Pro Ala Lys Arg Leu Glu Trp Val Ala Thr Ile Ser Ser Gly
65 70 75 80

agt agt acc atc tac tat gca gac aca gtg aag ggc cga ttc acc atc 288
Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe Thr Ile
85 90 95

tcc aga gac aat gcc aag aac acc ctg ttc ctg caa atg acc agt cta 336
Ser Arg Asp Asn Ala Lys Asn Thr Leu Phe Leu Gln Met Thr Ser Leu
100 105 110

agg tct gag gac aca gcc atg tat tac tgt gca agg aga tgg ttt ctt 384
Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg Arg Trp Phe Leu
115 120 125

gac tgc tgg ggc caa ggc acc act ctc aca gtc tcc tcg 423
Asp Cys Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
130 135 140

<210> 155
<211> 141
<212> PRT
<213> Mus musculus

<400> 155

Met Val Leu Ala Ser Ser Thr Thr Ser Ile His Thr Met Leu Leu Leu
1 5 10 15

Leu Leu Met Leu Ala Gln Pro Ala Met Ala Glu Val Lys Leu Val Glu
20 25 30

Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Arg Lys Leu Ser Cys
35 40 45

Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Thr Met Ser Trp Val Arg
50 55 60

Gln Thr Pro Ala Lys Arg Leu Glu Trp Val Ala Thr Ile Ser Ser Gly
65 70 75 80

Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe Thr Ile
85 90 95

Ser Arg Asp Asn Ala Lys Asn Thr Leu Phe Leu Gln Met Thr Ser Leu
100 105 110

Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg Arg Trp Phe Leu
115 120 125

Asp Cys Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
130 135 140

<210> 156
<211> 357
<212> DNA

<213> Mus musculus

<220>
<221> CDS
<222> (1)..(357)
<223>

<400> 156

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gat att gtg ctc acc caa tct cca gct tct ttg gct gtg tct cta ggg       48
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15

cag agt gtc acc atc tcc tgc aga gcc agt gaa agt gtt gaa tat tat       96
Gln Ser Val Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Glu Tyr Tyr
            20                  25                  30

ggc act agt tta atg cag tgg tac caa cag aaa cca gga cag cca ccc      144
Gly Thr Ser Leu Met Gln Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

aaa ctc ctc atc tat ggt gca tcc aac gta gaa tct ggg gtc cct gcc      192
Lys Leu Leu Ile Tyr Gly Ala Ser Asn Val Glu Ser Gly Val Pro Ala
    50                  55                  60

agg ttt agt ggc agt ggg tct ggg aca gac ttc agc ctc aac atc cat      240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
65                  70                  75                  80

cct gtg gag gag gat gat att gca atg tat ttc tgt cag caa agt agg      288
Pro Val Glu Glu Asp Asp Ile Ala Met Tyr Phe Cys Gln Gln Ser Arg
                85                  90                  95

aag gtt ccg tgg acg ttc ggt gga ggc acc aag ctg gaa ata aag gac      336
Lys Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Asp
            100                 105                 110
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tac aag gat gac gac gat aag 357
Tyr Lys Asp Asp Asp Asp Lys
115

<210> 157
<211> 119
<212> PRT
<213> Mus musculus

<400> 157

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Gln Ser Val Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Glu Tyr Tyr
20 25 30

Gly Thr Ser Leu Met Gln Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Gly Ala Ser Asn Val Glu Ser Gly Val Pro Ala
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
65 70 75 80

Pro Val Glu Glu Asp Asp Ile Ala Met Tyr Phe Cys Gln Gln Ser Arg
85 90 95

Lys Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Asp
100 105 110

Tyr Lys Asp Asp Asp Asp Lys
115

<210> 158
<211> 432
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(432)
<223>

<400> 158
atg gtt ctt gcc agc tct acc acc agc atc cac acc atg ctg ctc ctg 48
Met Val Leu Ala Ser Ser Thr Thr Ser Ile His Thr Met Leu Leu Leu
1 5 10 15

ctc ctg atg ctg gcc cag ccg gcc atg gcg cag gtt cag ctc cag caa 96
Leu Leu Met Leu Ala Gln Pro Ala Met Ala Gln Val Gln Leu Gln Gln
20 25 30

tct gga cct gag ctg gtg aag cct ggg gcc tca gtg aag att tcc tgc 144
Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys
35 40 45

aag gct tct ggc tat gca ttc agt agc tcc tgg atg aac tgg atg aag 192
Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp Met Lys
50 55 60

cag agg cct gga aag ggt ctt gag tgg att ggg cgg att tat cct gga 240

Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly
65 70 75 80

gat gga gat act aac tac aat ggg aag ttc aag ggc aag gcc aca ctg 288
Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu
85 90 95

act gca gac aaa tcc tcc agc aca gcc tac atg caa ctc agc agc ctg 336
Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu
100 105 110

aca tct gag gac tct gcg gtc tac ttc tgt gca aga gcg agg aaa act 384
Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Ala Arg Lys Thr
115 120 125

tcc tgg ttt gct tac tgg ggc caa ggg act ctg gtc act gtc tct gcg 432
Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
130 135 140

<210> 159
<211> 144
<212> PRT
<213> Mus musculus

<400> 159

Met Val Leu Ala Ser Ser Thr Thr Ser Ile His Thr Met Leu Leu Leu
1 5 10 15

Leu Leu Met Leu Ala Gln Pro Ala Met Ala Gln Val Gln Leu Gln Gln
20 25 30

Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys

35 40 45

Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser Trp Met Asn Trp Met Lys
50 55 60

Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly
65 70 75 80

Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu
85 90 95

Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu
100 105 110

Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Ala Arg Lys Thr
115 120 125

Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
130 135 140

<210> 160
<211> 345
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(345)
<223>

<400> 160

gac att gtg ttg aca cag tct caa aaa ttc atg tcc aca tca gta gga 48
Asp Ile Val Leu Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

gac agg gtc agc atc agc tgc aag gcc agt cag aat gtg ggt aat att 96
Asp Arg Val Ser Ile Ser Cys Lys Ala Ser Gln Asn Val Gly Asn Ile
20 25 30

ata gcc tgg tat caa cag aaa cca ggg caa tct cct aaa gca ctg att 144
Ile Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
35 40 45

tac ttg gca tcc tac cgg tac agt gga gtc cct gat cgc ttc aca ggc 192
Tyr Leu Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

agt gga tct ggg aca gat ttc act ctc acc att agt aat gtg cag tct 240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
65 70 75 80

gaa gac ttg gca gag tat ttc tgt cag caa tat agc agc tct ccg ctc 288
Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Ser Ser Ser Pro Leu
85 90 95

acg ttc ggt gct ggg acc aag ctg gaa ata aag gac tac aag gat gac 336
Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Asp Tyr Lys Asp Asp
100 105 110

gac gat aag 345
Asp Asp Lys
115

<210> 161

<211> 115
<212> PRT
<213> Mus musculus

<400> 161

Asp Ile Val Leu Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Ile Ser Cys Lys Ala Ser Gln Asn Val Gly Asn Ile
20 25 30

Ile Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
35 40 45

Tyr Leu Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
65 70 75 80

Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Ser Ser Ser Pro Leu
85 90 95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Asp Tyr Lys Asp Asp
100 105 110

Asp Asp Lys
115

<210> 162
<211> 116
<212> PRT
<213> Mus musculus

<400> 162

Asp Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Ser Leu Ser Leu Thr Cys Thr Val Thr Gly Tyr Ser Ile Thr Ser Asp
20 25 30

Tyr Ala Trp Ser Trp Ile Arg Gln Leu Pro Gly Asn Lys Leu Glu Trp
35 40 45

Met Gly Tyr Ile Thr Tyr Ser Gly Tyr Ser Ile Tyr Asn Pro Ser Leu
50 55 60

Lys Ser Arg Ile Ser Ile Ser Arg Asp Thr Ser Lys Asn Gln Leu Phe
65 70 75 80

Leu Gln Leu Asn Ser Val Thr Thr Glu Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Val Gly Gly Tyr Asp Asn Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
100 105 110

Thr Val Ser Ser
115

<210> 163
<211> 108
<212> PRT
<213> Mus musculus

<400> 163

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Leu Thr Cys Ser Ala Ser Ser Ser Val Ser Ser Ser
20 25 30

His Leu Tyr Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Trp
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Asn Met Glu
65 70 75 80

Thr Glu Asp Ala Ala Ser Tyr Phe Cys His Gln Trp Ser Ser Tyr Pro
85 90 95

Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 164
<211> 1924
<212> DNA
<213> Macaca fascicularis

<220>
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<222> (11)..(1918)
<223>

<400> 164
gaattccacc atg ccc tcc tgg gcc ctc ttc atg gtc acc tcc tgc ctc 49
Met Pro Ser Trp Ala Leu Phe Met Val Thr Ser Cys Leu
1 5 10

ctc ctg gcc cct caa aac ctg gcc caa gtc agc agc caa gat gtc tcc 97
Leu Leu Ala Pro Gln Asn Leu Ala Gln Val Ser Ser Gln Asp Val Ser
15 20 25

ttg ctg gcc tcg gac tca gag ccc ctg aag tgt ttc tcc cga aca ttt 145
Leu Leu Ala Ser Asp Ser Glu Pro Leu Lys Cys Phe Ser Arg Thr Phe
30 35 40 45

gag gac ctc act tgc ttc tgg gat gag gaa gag gca gca ccc agt ggg 193
Glu Asp Leu Thr Cys Phe Trp Asp Glu Glu Glu Ala Ala Pro Ser Gly
50 55 60

aca tac cag ctg ctg tat gcc tac ccg ggg gag aag ccc cgt gcc tgc 241
Thr Tyr Gln Leu Leu Tyr Ala Tyr Pro Gly Glu Lys Pro Arg Ala Cys
65 70 75

ccc ctg agt tct cag agc gtg ccc cgc ttt gga acc cga tac gtg tgc 289

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Pro Leu Ser Ser Gln Ser Val Pro Arg Phe Gly Thr Arg Tyr Val Cys
        80                  85                  90

cag ttt cca gcc cag gaa gaa gtg cgt ctc ttc tct ccg ctg cac ctc      337
Gln Phe Pro Ala Gln Glu Glu Val Arg Leu Phe Ser Pro Leu His Leu
    95                  100                 105

tgg gtg aag aat gtg ttc cta aac cag act cag att cag cga gtc ctc      385
Trp Val Lys Asn Val Phe Leu Asn Gln Thr Gln Ile Gln Arg Val Leu
110                 115                 120                 125

ttt gtg gac agt gta ggc ctg ccg gct ccc ccc agt atc atc aag gcc      433
Phe Val Asp Ser Val Gly Leu Pro Ala Pro Pro Ser Ile Ile Lys Ala
                130                 135                 140

atg ggt ggg agc cag cca ggg gaa ctt cag atc agc tgg gag gcc cca      481
Met Gly Gly Ser Gln Pro Gly Glu Leu Gln Ile Ser Trp Glu Ala Pro
            145                 150                 155

gct cca gaa atc agt gat ttc ctg agg tac gaa ctc cgc tat ggc ccc      529
Ala Pro Glu Ile Ser Asp Phe Leu Arg Tyr Glu Leu Arg Tyr Gly Pro
        160                 165                 170

aaa gat ctc aag aac tcc act ggt ccc acg gtc ata cag ttg atc gcc      577
Lys Asp Leu Lys Asn Ser Thr Gly Pro Thr Val Ile Gln Leu Ile Ala
    175                 180                 185

aca gaa acc tgc tgc cct gct ctg cag agg cca cac tca gcc tct gct      625
Thr Glu Thr Cys Cys Pro Ala Leu Gln Arg Pro His Ser Ala Ser Ala
190                 195                 200                 205

ctg gac cag tct cca tgt gct cag ccc aca atg ccc tgg caa gat gga      673
Leu Asp Gln Ser Pro Cys Ala Gln Pro Thr Met Pro Trp Gln Asp Gly
                210                 215                 220

cca aag cag acc tcc cca act aga gaa gct tca gct ctg aca gca gtg      721
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Pro Lys Gln Thr Ser Pro Thr Arg Glu Ala Ser Ala Leu Thr Ala Val
            225                 230                 235

ggt gga agc tgc ctc atc tca gga ctc cag cct ggc aac tcc tac tgg     769
Gly Gly Ser Cys Leu Ile Ser Gly Leu Gln Pro Gly Asn Ser Tyr Trp
        240                 245                 250

ctg cag ctg cgc agc gaa cct gat ggg atc tcc ctc ggt ggc tcc tgg     817
Leu Gln Leu Arg Ser Glu Pro Asp Gly Ile Ser Leu Gly Gly Ser Trp
    255                 260                 265

gga tcc tgg tcc ctc cct gtg act gtg gac ctg cct gga gat gca gtg     865
Gly Ser Trp Ser Leu Pro Val Thr Val Asp Leu Pro Gly Asp Ala Val
270                 275                 280                 285

gca att gga ctg caa tgc ttt acc ttg gac ctg aag aat gtt acc tgt     913
Ala Ile Gly Leu Gln Cys Phe Thr Leu Asp Leu Lys Asn Val Thr Cys
                290                 295                 300

caa tgg cag caa gag gac cat gct agt tcc caa ggt ttc ttc tac cac     961
Gln Trp Gln Gln Glu Asp His Ala Ser Ser Gln Gly Phe Phe Tyr His
            305                 310                 315

agc agg gca cgg tgc tgc ccc aga gac agg tac ccc atc tgg gag gac     1009
Ser Arg Ala Arg Cys Cys Pro Arg Asp Arg Tyr Pro Ile Trp Glu Asp
        320                 325                 330

tgt gaa gag gaa gag aaa aca aat cca gga tta cag acc cca cag ttc     1057
Cys Glu Glu Glu Glu Lys Thr Asn Pro Gly Leu Gln Thr Pro Gln Phe
    335                 340                 345

tct cgc tgc cac ttc aag tca cga aat gac agc gtt att cac atc ctt     1105
Ser Arg Cys His Phe Lys Ser Arg Asn Asp Ser Val Ile His Ile Leu
350                 355                 360                 365

gtg gag gtg acc aca gcc ctg ggt gct gtt cac agt tac ctg ggc tcc     1153
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Val Glu Val Thr Thr Ala Leu Gly Ala Val His Ser Tyr Leu Gly Ser
                370                 375                 380

cct ttc tgg atc cac cag gct gtg cgc ctc ccc acc cca aac ttg cac    1201
Pro Phe Trp Ile His Gln Ala Val Arg Leu Pro Thr Pro Asn Leu His
            385                 390                 395

tgg agg gag atc tcc agc ggg cat ctg gaa ttg gag tgg cag cac cca    1249
Trp Arg Glu Ile Ser Ser Gly His Leu Glu Leu Glu Trp Gln His Pro
        400                 405                 410

tca tcc tgg gca gcc caa gag acc tgc tat caa ctc cga tac aca gga    1297
Ser Ser Trp Ala Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly
    415                 420                 425

gaa ggc cat cag gac tgg aag gtg ctg gag ccg cct ctc ggg gcc cga    1345
Glu Gly His Gln Asp Trp Lys Val Leu Glu Pro Pro Leu Gly Ala Arg
430                 435                 440                 445

gga ggg acc ctg gag ctg cgc ccg cga tct cgc tac cgt tta cag ctg    1393
Gly Gly Thr Leu Glu Leu Arg Pro Arg Ser Arg Tyr Arg Leu Gln Leu
                450                 455                 460

cgc gcc agg ctc aat ggc ccc acc tac caa ggt ccc tgg agc tcg tgg    1441
Arg Ala Arg Leu Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ser Trp
            465                 470                 475

tcg gac cca gct agg gtg gag acc gcc acc gag acc gcc tgg att tcc    1489
Ser Asp Pro Ala Arg Val Glu Thr Ala Thr Glu Thr Ala Trp Ile Ser
        480                 485                 490

ttg gtg acc gct ctg ctg cta gtg ctg ggc ctc agc gcc gtc ctg ggc    1537
Leu Val Thr Ala Leu Leu Leu Val Leu Gly Leu Ser Ala Val Leu Gly
    495                 500                 505

ctg ctg ctg ctg agg tgg cag ttt cct gca cac tac agg aga ctg agg    1585
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Leu Leu Leu Leu Arg Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg
510 515 520 525

cat gcc ctg tgg ccc tca ctt cca gat ctg cac cga gtc cta ggc cag 1633
His Ala Leu Trp Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln
530 535 540

tac ctt agg gac act gca gcc ctg agt ccg ccc aag gcc aca gtc tca 1681
Tyr Leu Arg Asp Thr Ala Ala Leu Ser Pro Pro Lys Ala Thr Val Ser
545 550 555

gat acc tgt gaa gaa gtg gaa ccc agc ctc ctt gaa atc ctc ccc aag 1729
Asp Thr Cys Glu Glu Val Glu Pro Ser Leu Leu Glu Ile Leu Pro Lys
560 565 570

tcc tca gag agg act cct ttg ccc ctg tgt tcc tcc cag tcc cag atg 1777
Ser Ser Glu Arg Thr Pro Leu Pro Leu Cys Ser Ser Gln Ser Gln Met
575 580 585

gac tac cga aga ttg cag cct tct tgc ctg ggg acc atg ccc ctg tct 1825
Asp Tyr Arg Arg Leu Gln Pro Ser Cys Leu Gly Thr Met Pro Leu Ser
590 595 600 605

gtg tgc cca ccc atg gct gag tca ggg tcc tgc tgt acc acc cac att 1873
Val Cys Pro Pro Met Ala Glu Ser Gly Ser Cys Cys Thr Thr His Ile
610 615 620

gcc aac cat tcc tac cta cca cta agc tat tgg cag cag cct tga 1918
Ala Asn His Ser Tyr Leu Pro Leu Ser Tyr Trp Gln Gln Pro
625 630 635

gtcgac 1924

<210> 165
<211> 635

<212> PRT
<213> Macaca fascicularis

<400> 165

Met Pro Ser Trp Ala Leu Phe Met Val Thr Ser Cys Leu Leu Leu Ala
1 5 10 15

Pro Gln Asn Leu Ala Gln Val Ser Ser Gln Asp Val Ser Leu Leu Ala
20 25 30

Ser Asp Ser Glu Pro Leu Lys Cys Phe Ser Arg Thr Phe Glu Asp Leu
35 40 45

Thr Cys Phe Trp Asp Glu Glu Glu Ala Ala Pro Ser Gly Thr Tyr Gln
50 55 60

Leu Leu Tyr Ala Tyr Pro Gly Glu Lys Pro Arg Ala Cys Pro Leu Ser
65 70 75 80

Ser Gln Ser Val Pro Arg Phe Gly Thr Arg Tyr Val Cys Gln Phe Pro
85 90 95

Ala Gln Glu Glu Val Arg Leu Phe Ser Pro Leu His Leu Trp Val Lys
100 105 110

Asn Val Phe Leu Asn Gln Thr Gln Ile Gln Arg Val Leu Phe Val Asp
115 120 125

Ser Val Gly Leu Pro Ala Pro Pro Ser Ile Ile Lys Ala Met Gly Gly
130 135 140

Ser Gln Pro Gly Glu Leu Gln Ile Ser Trp Glu Ala Pro Ala Pro Glu
145 150 155 160

Ile Ser Asp Phe Leu Arg Tyr Glu Leu Arg Tyr Gly Pro Lys Asp Leu
165 170 175

Lys Asn Ser Thr Gly Pro Thr Val Ile Gln Leu Ile Ala Thr Glu Thr
180 185 190

Cys Cys Pro Ala Leu Gln Arg Pro His Ser Ala Ser Ala Leu Asp Gln
195 200 205

Ser Pro Cys Ala Gln Pro Thr Met Pro Trp Gln Asp Gly Pro Lys Gln
210 215 220

Thr Ser Pro Thr Arg Glu Ala Ser Ala Leu Thr Ala Val Gly Gly Ser
225 230 235 240

Cys Leu Ile Ser Gly Leu Gln Pro Gly Asn Ser Tyr Trp Leu Gln Leu
245 250 255

Arg Ser Glu Pro Asp Gly Ile Ser Leu Gly Gly Ser Trp Gly Ser Trp
260 265 270

Ser Leu Pro Val Thr Val Asp Leu Pro Gly Asp Ala Val Ala Ile Gly
275 280 285

Leu Gln Cys Phe Thr Leu Asp Leu Lys Asn Val Thr Cys Gln Trp Gln
290 295 300

Gln Glu Asp His Ala Ser Ser Gln Gly Phe Phe Tyr His Ser Arg Ala
305 310 315 320

Arg Cys Cys Pro Arg Asp Arg Tyr Pro Ile Trp Glu Asp Cys Glu Glu
325 330 335

Glu Glu Lys Thr Asn Pro Gly Leu Gln Thr Pro Gln Phe Ser Arg Cys
340 345 350

His Phe Lys Ser Arg Asn Asp Ser Val Ile His Ile Leu Val Glu Val
355 360 365

Thr Thr Ala Leu Gly Ala Val His Ser Tyr Leu Gly Ser Pro Phe Trp
370 375 380

Ile His Gln Ala Val Arg Leu Pro Thr Pro Asn Leu His Trp Arg Glu
385 390 395 400

Ile Ser Ser Gly His Leu Glu Leu Glu Trp Gln His Pro Ser Ser Trp
405 410 415

Ala Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly Glu Gly His
420 425 430

Gln Asp Trp Lys Val Leu Glu Pro Pro Leu Gly Ala Arg Gly Gly Thr
435 440 445

Leu Glu Leu Arg Pro Arg Ser Arg Tyr Arg Leu Gln Leu Arg Ala Arg
450 455 460

Leu Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ser Trp Ser Asp Pro
465 470 475 480

Ala Arg Val Glu Thr Ala Thr Glu Thr Ala Trp Ile Ser Leu Val Thr
485 490 495

Ala Leu Leu Leu Val Leu Gly Leu Ser Ala Val Leu Gly Leu Leu Leu
500 505 510

Leu Arg Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu
515 520 525

Trp Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg
530 535 540

Asp Thr Ala Ala Leu Ser Pro Pro Lys Ala Thr Val Ser Asp Thr Cys
545 550 555 560

Glu Glu Val Glu Pro Ser Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu
565 570 575

Arg Thr Pro Leu Pro Leu Cys Ser Ser Gln Ser Gln Met Asp Tyr Arg
580 585 590

Arg Leu Gln Pro Ser Cys Leu Gly Thr Met Pro Leu Ser Val Cys Pro
595 600 605

Pro Met Ala Glu Ser Gly Ser Cys Cys Thr Thr His Ile Ala Asn His
610 615 620

Ser Tyr Leu Pro Leu Ser Tyr Trp Gln Gln Pro
625 630 635

<210> 166
<211> 24
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<220>
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<400> 166
caggggccag tggatagact gatg 24

<210> 167
<211> 23
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<220>
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<400> 167
gctcactgga tggtgggaag atg 23

<210> 168
<211> 30
<212> DNA
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<220>
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<400> 168
tagaattcca ccatggaatg gcctttgatc 30

<210> 169
<211> 56
<212> DNA
<213> Artificial

<220>
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<400> 169
agcctgagtc atcacaatat ccgatccgcc tccacctgca gagacagtga ccagag 56

<210> 170
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<212> DNA

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<220>
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actctggtca ctgtctctgc aggtggaggc ggatcggata ttgtgatgac tcaggc 56

<210> 171
<211> 60
<212> DNA
<213> Artificial

<220>
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<400> 171
attgcggccg cttatcactt atcgtcgtca tccttgtagt cttttatttc cagcttggtc 60

<210> 172
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<212> PRT
<213> Artificial

<220>
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<400> 172

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> 173

<211> 85
<212> DNA
<213> Artificial

<220>
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<400> 173
tagaattcca ccatggaatg gcctttgatc tttctcttcc tcctgtcagg aactgcaggt 60

gtccactccc aggttcagct gcagc 85

<210> 174
<211> 82
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 174
tggtcactgt ctctgcaggt ggtggtggtt cgggtggtgg tggttcgggt ggtggcggat 60

cggatattgt gatgactcag gc 82

<210> 175
<211> 82
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 175

tgagtcatca caatatccga tccgccacca cccgaaccac caccacccga accaccacca 60

cctgcagaga cagtgaccag ag 82

<210> 176
<211> 25
<212> DNA
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<220>
<223> an artificially synthesized primer sequence

<400> 176
caggttcagc tgcagcagtc tggac 25

<210> 177
<211> 81
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 177
gctgcagctg aacctgcgat ccaccgcctc ccgaaccacc accacccgat ccaccacctc 60

cttttatttc agcttggtc c 81

<210> 178
<211> 38
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 178
gcccagccgg ccatggcgga kgtrmagctt caggagtc 38

<210> 179
<211> 38
<212> DNA
<213> Artificial

<220>
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<400> 179
gcccagccgg ccatggcgga ggtbcagctb cagcagtc 38

<210> 180
<211> 38
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 180
gcccagccgg ccatggcgca ggtgcagctg aagsastc 38

<210> 181
<211> 38
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<213> Artificial

<220>
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<400> 181
gcccagccgg ccatggcgga ggtccarctg caacartc 38

<210> 182
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<220>
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<400> 182
gcccagccgg ccatggcgca ggtycagctb cagcartc 38

<210> 183
<211> 38
<212> DNA
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<220>
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<400> 183
gcccagccgg ccatggcgca ggtycarctg cagcagtc 38

<210> 184
<211> 38
<212> DNA
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<400> 184
gcccagccgg ccatggcgca ggtccacgtg aagcagtc 38

<210> 185
<211> 38
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 185
gcccagccgg ccatggcgga ggtgaasstg gtggaatc 38

<210> 186
<211> 38
<212> DNA
<213> Artificial

<220>
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<400> 186
gcccagccgg ccatggcgga vgtgawgytg gtggagtc 38

<210> 187
<211> 38
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<220>
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<400> 187
gcccagccgg ccatggcgga ggtgcagskg gtggagtc 38

<210> 188
<211> 38
<212> DNA
<213> Artificial

<220>
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<400> 188
gcccagccgg ccatggcgga kgtgcamctg gtggagtc 38

<210> 189
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<400> 189
gcccagccgg ccatggcgga ggtgaagctg atggartc 38

<210> 190
<211> 38
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<400> 190
gcccagccgg ccatggcgga ggtgcarctt gttgagtc 38

<210> 191
<211> 38
<212> DNA
<213> Artificial

<220>
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<400> 191
gcccagccgg ccatggcgga rgtraagctt ctcgagtc 38

<210> 192
<211> 38
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 192
gcccagccgg ccatggcgga agtgaarstt gaggagtc 38

<210> 193
<211> 40
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

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gcccagccgg ccatggcgca ggttactctr aaagwgtstg 40

<210> 194
<211> 38
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<400> 194
gcccagccgg ccatggcgca ggtccaactv cagcarcc 38

<210> 195
<211> 38
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 195
gcccagccgg ccatggcgga tgtgaacttg gaagtgtc 38

<210> 196
<211> 38
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 196
gcccagccgg ccatggcgga ggtgaaggtc atcgagtc 38

<210> 197
<211> 36
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

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ggagccgccg ccgcccgagg aaacggtgac cgtggt 36

<210> 198
<211> 36
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 198
ggagccgccg ccgcccgagg agactgtgag agtggt 36

<210> 199
<211> 36
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 199
ggagccgccg ccgcccgcag agacagtgac cagagt 36

<210> 200
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<212> DNA
<213> Artificial

<220>
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<400> 200
ggagccgccg ccgcccgagg agacggtgac tgaggt 36

<210> 201
<211> 35
<212> DNA
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<220>
<223> an artificially synthesized primer sequence

<400> 201
ggcggcggcg gctccgayat ccagctgact cagcc 35

<210> 202
<211> 35
<212> DNA
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<220>
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ggcggcggcg gctccgayat tgttctcwcc cagtc 35

<210> 203
<211> 35
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<220>
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ggcggcggcg gctccgayat tgtgmtmact cagtc 35

<210> 204
<211> 35
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<213> Artificial

<220>
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<400> 204
ggcggcggcg gctccgayat tgtgytraca cagtc 35

<210> 205
<211> 35
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<220>
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ggcggcggcg gctccgayat tgtratgacm cagtc 35

<210> 206
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ggcggcggcg gctccgayat tmagatramc cagtc 35

<210> 207
<211> 35
<212> DNA
<213> Artificial

<220>
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<400> 207
ggcggcggcg gctccgayat tcagatgayd cagtc 35

<210> 208
<211> 35
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 208
ggcggcggcg gctccgayat ycagatgaca cagac 35

<210> 209
<211> 35
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 209
ggcggcggcg gctccgayat tgttctcawc cagtc 35

<210> 210
<211> 35
<212> DNA
<213> Artificial

<220>
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<400> 210
ggcggcggcg gctccgayat tgwgctsacc caatc 35

<210> 211
<211> 35
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 211
ggcggcggcg gctccgayat tstratgacc cartc 35

<210> 212
<211> 35
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 212
ggcggcggcg gctccgayrt tktgatgacc carac 35

<210> 213
<211> 35
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<213> Artificial

<220>
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<400> 213
ggcggcggcg gctccgayat tgtgatgacb cagkc 35

<210> 214
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<220>
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<400> 214
ggcggcggcg gctccgayat tgtgataacy cagga 35

<210> 215
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<220>
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<400> 215
ggcggcggcg gctccgayat tgtgatgacc cagwt 35

<210> 216
<211> 35
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 216
ggcggcggcg gctccgayat tgtgatgaca caacc 35

<210> 217
<211> 35
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 217
ggcggcggcg gctccgayat tttgctgact cagtc 35

<210> 218
<211> 38
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 218
ggcggcggcg gctccgatgc tgttgtgact caggaatc 38

<210> 219
<211> 36
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 219
ggaattcggc ccccgaggcc ttgatttcca gcttgg 36

<210> 220
<211> 36
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 220
ggaattcggc ccccgaggcc tttatttcca gcttgg 36

<210> 221
<211> 36
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 221
ggaattcggc ccccgaggcc tttatttcca actttg 36

<210> 222
<211> 36
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 222
ggaattcggc ccccgaggcc ttcagctcca gcttgg 36

<210> 223
<211> 39
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized primer sequence

<400> 223
ggaattcggc ccccgaggcc cctaggacag tcagtttgg 39

<210> 224
<211> 27
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 224
ttactcgcgg cccagccggc catggcg 27

<210> 225
<211> 17
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 225
ggaattcggc ccccgag 17

<210> 226
<211> 20
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 226
tcacttacag gctctctact 20

<210> 227
<211> 20
<212> DNA
<213> Artificial

<220>
<223> an artificially synthesized sequence

<400> 227
caggtggggt ctttcattcc 20

<210> 228
<211> 354
<212> DNA
<213> Homo sapiens

<400> 228
caggtgcagc tggtgcagtc tggacctgag gtgaagaagc ctggggcctc agtgaaggtc 60

tcctgcaagg cttctggata caccttcacc aactcctgga tgaactgggt gaggcagagg 120

cctggaaagg gtcttgagtg gatgggacgg atttatcctg gagatggaga aactatctac 180

aatgggaaat tcagggtcag agtcacgatt accgcggacg aatccacgag cacagcctac 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaggctat 300

gatgattact cgtttgctta ctggggccag ggaaccacgg tcaccgtctc ttca 354

<210> 229
<211> 118
<212> PRT
<213> Homo sapiens

<400> 229

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn Gly Lys Phe
50 55 60

Arg Val Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

<210> 230
<211> 30
<212> PRT
<213> Homo sapiens

<400> 230

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
20 25 30

<210> 231
<211> 5
<212> PRT
<213> Homo sapiens

<400> 231

Asn Ser Trp Met Asn
1 5

<210> 232
<211> 14
<212> PRT
<213> Homo sapiens

<400> 232

Trp Val Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Met Gly
1 5 10

<210> 233
<211> 17
<212> PRT
<213> Homo sapiens

<400> 233

Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn Gly Lys Phe Arg
1 5 10 15

Val

<210> 234
<211> 32
<212> PRT
<213> Homo sapiens

<400> 234

Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr Met Glu
1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 235
<211> 9
<212> PRT
<213> Homo sapiens

<400> 235

Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr
1 5

<210> 236
<211> 11
<212> PRT
<213> Homo sapiens

<400> 236

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
1 5 10

<210> 237
<211> 336
<212> DNA
<213> Homo sapiens

<400> 237
gatattgtga tgactcagtc tgcactctcc ctgcccgtca cccctggaga gccggcctcc 60

atctcctgca ggtctagtaa gagtctcctg catagtaatg gcaacactta cttgtattgg 120

ttccagcaga agccagggca gtctccacag ctcctgatct atcggatgtc caaccttgcc 180

tcaggggtcc ctgacaggtt cagtggcagt ggatcaggca cagcttttac actgaaaatc 240

agcagagtgg aggctgagga tgttggggtt tattactgca tgcaacatat agaatatcct 300

tttacgttcg gccaagggac caaactggaa atcaaa 336

<210> 238
<211> 112
<212> PRT
<213> Homo sapiens

<400> 238

Asp Ile Val Met Thr Gln Ser Ala Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Gln Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Ile Glu Tyr Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 239
<211> 23
<212> PRT
<213> Homo sapiens

<400> 239

Asp Ile Val Met Thr Gln Ser Ala Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys
20

<210> 240
<211> 16
<212> PRT
<213> Homo sapiens

<400> 240

Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
1 5 10 15

<210> 241
<211> 15
<212> PRT
<213> Homo sapiens

<400> 241

Trp Phe Gln Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
1 5 10 15

<210> 242
<211> 7
<212> PRT
<213> Homo sapiens

<400> 242

Arg Met Ser Asn Leu Ala Ser
1 5

<210> 243
<211> 32
<212> PRT
<213> Homo sapiens

<400> 243

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr
1 5 10 15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
20 25 30

<210> 244
<211> 9
<212> PRT
<213> Homo sapiens

<400> 244

Met Gln His Ile Glu Tyr Pro Phe Thr
1 5

<210> 245
<211> 10
<212> PRT
<213> Homo sapiens

<400> 245

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 246
<211> 1924
<212> DNA
<213> Homo sapiens

<400> 246
gaattccacc atgccctcct gggccctctt catggtcacc tcctgcctcc tcctggcccc 60

tcaaaacctg gcccaagtca gcagccaaga tgtctccttg ctggcatcag actcagagcc 120

cctgaagtgt ttctcccgaa catttgagga cctcacttgc ttctgggatg aggaagaggc 180

agcgcccagt gggacatacc agctgctgta tgcctacccg cgggagaagc cccgtgcttg 240

ccccctgagt tcccagagca tgccccactt tggaacccga tacgtgtgcc agtttccaga 300

ccaggaggaa gtgcgtctct tctttccgct gcacctctgg gtgaagaatg tgttcctaaa 360

ccagactcgg actcagcgag tcctctttgt ggacagtgta ggcctgccgg ctcccccccag 420

tatcatcaag gccatgggtg ggagccagcc aggggaactt cagatcagct gggaggagcc 480

agctccagaa atcagtgatt tcctgaggta cgaactccgc tatggcccca gagatcccaa 540

gaactccact ggtcccacgg tcatacagct gattgccaca gaaacctgct gccctgctct 600

gcagagacct cactcagcct ctgctctgga ccagtctcca tgtgctcagc ccacaatgcc 660

ctggcaagat ggaccaaagc agacctcccc aagtagagaa gcttcagctc tgacagcaga 720

gggtggaagc tgcctcatct caggactcca gcctggcaac tcctactggc tgcagctgcg 780

cagcgaacct gatgggatct ccctcggtgg ctcctgggga tcctggtccc tccctgtgac 840

tgtggacctg cctggagatg cagtggcact tggactgcaa tgctttacct tggacctgaa 900

gaatgttacc tgtcaatggc agcaacagga ccatgctagc tcccaaggct tcttctacca 960

cagcagggca cggtgctgcc ccagagacag gtaccccatc tgggagaact gcgaagagga 1020

agagaaaaca aatccaggac tacagacccc acagttctct cgctgccact tcaagtcacg 1080

aaatgacagc attattcaca tccttgtgga ggtgaccaca gccccgggta ctgttcacag 1140

ctacctgggc tccccttttct ggatccacca ggctgtgcgc ctccccaccc caaacttgca 1200

ctggagggag atctccagtg ggcatctgga attggagtgg cagcacccat cgtcctgggc 1260

agcccaagag acctgttatc aactccgata cacaggagaa ggccatcagg actggaaggt 1320

gctggagccg cctctcgggg cccgaggagg gaccctggag ctgcgcccgc gatctcgcta 1380

ccgtttacag ctgcgcgcca ggctcaacgg ccccacctac caaggtccct ggagctcgtg 1440

gtcggaccca actagggtgg agaccgccac cgagaccgcc tggatctcct tggtgaccgc 1500

tctgcatcta gtgctgggcc tcagcgccgt cctgggcctg ctgctgctga ggtggcagtt 1560

tcctgcacac tacaggagac tgaggcatgc cctgtggccc tcacttccag acctgcaccg 1620

ggtcctaggc cagtacctta gggacactgc agccctgagc ccgcccaagg ccacagtctc 1680

agatacctgt gaagaagtgg aacccagcct ccttgaaatc ctccccaagt cctcagagag 1740

gactcctttg cccctgtgtt cctcccaggc ccagatggac taccgaagat tgcagccttc 1800

ttgcctgggg accatgcccc tgtctgtgtg cccacccatg gctgagtcag ggtcctgctg 1860

taccacccac attgccaacc attcctacct accactaagc tattggcagc agccttgagt 1920

cgac 1924

<210> 247
<211> 1924
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (11)..(1918)
<223>

<400> 247
gaattccacc atg ccc tcc tgg gcc ctc ttc atg gtc acc tcc tgc ctc 49
Met Pro Ser Trp Ala Leu Phe Met Val Thr Ser Cys Leu
1 5 10

ctc ctg gcc cct caa aac ctg gcc caa gtc agc agc caa gat gtc tcc 97
Leu Leu Ala Pro Gln Asn Leu Ala Gln Val Ser Ser Gln Asp Val Ser
15 20 25

ttg ctg gca tca gac tca gag ccc ctg aag tgt ttc tcc cga aca ttt 145
Leu Leu Ala Ser Asp Ser Glu Pro Leu Lys Cys Phe Ser Arg Thr Phe
30 35 40 45

gag gac ctc act tgc ttc tgg gat gag gaa gag gca gcg ccc agt ggg 193
Glu Asp Leu Thr Cys Phe Trp Asp Glu Glu Glu Ala Ala Pro Ser Gly
50 55 60

aca tac cag ctg ctg tat gcc tac ccg cgg gag aag ccc cgt gct tgc 241
Thr Tyr Gln Leu Leu Tyr Ala Tyr Pro Arg Glu Lys Pro Arg Ala Cys
65 70 75

ccc ctg agt tcc cag agc atg ccc cac ttt gga acc cga tac gtg tgc 289
Pro Leu Ser Ser Gln Ser Met Pro His Phe Gly Thr Arg Tyr Val Cys
80 85 90

cag ttt cca gac cag gag gaa gtg cct ctc ttc ttt ccg ctg cac ctc 337
Gln Phe Pro Asp Gln Glu Glu Val Pro Leu Phe Phe Pro Leu His Leu
95 100 105

tgg gtg aag aat gtg ttc cta aac cag act cgg act cag cga gtc ctc 385
Trp Val Lys Asn Val Phe Leu Asn Gln Thr Arg Thr Gln Arg Val Leu
110 115 120 125

ttt gtg gac agt gta ggc ctg ccg gct ccc ccc agt atc atc aag gcc 433
Phe Val Asp Ser Val Gly Leu Pro Ala Pro Pro Ser Ile Ile Lys Ala
130 135 140

atg ggt ggg agc cag cca ggg gaa ctt cag atc agc tgg gag gag cca 481
Met Gly Gly Ser Gln Pro Gly Glu Leu Gln Ile Ser Trp Glu Glu Pro
145 150 155

gct cca gaa atc agt gat ttc ctg agg tac gaa ctc cgc tat ggc ccc 529
Ala Pro Glu Ile Ser Asp Phe Leu Arg Tyr Glu Leu Arg Tyr Gly Pro
160 165 170

aga gat ccc aag aac tcc act ggt ccc acg gtc ata cag ctg att gcc 577
Arg Asp Pro Lys Asn Ser Thr Gly Pro Thr Val Ile Gln Leu Ile Ala
175 180 185

aca gaa acc tgc tgc cct gct ctg cag aga cct cac tca gcc tct gct 625
Thr Glu Thr Cys Cys Pro Ala Leu Gln Arg Pro His Ser Ala Ser Ala
190 195 200 205

ctg gac cag tct cca tgt gct cag ccc aca atg ccc tgg caa gat gga 673
Leu Asp Gln Ser Pro Cys Ala Gln Pro Thr Met Pro Trp Gln Asp Gly
210 215 220

cca aag cag acc tcc cca agt aga gaa gct tca gct ctg aca gca gag 721
Pro Lys Gln Thr Ser Pro Ser Arg Glu Ala Ser Ala Leu Thr Ala Glu
225 230 235

ggt gga agc tgc ctc atc tca gga ctc cag cct ggc aac tcc tac tgg 769
Gly Gly Ser Cys Leu Ile Ser Gly Leu Gln Pro Gly Asn Ser Tyr Trp
240 245 250

ctg cag ctg cgc agc gaa cct gat ggg atc tcc ctc ggt ggc tcc tgg 817
Leu Gln Leu Arg Ser Glu Pro Asp Gly Ile Ser Leu Gly Gly Ser Trp
255 260 265

gga tcc tgg tcc ctc cct gtg act gtg gac ctg cct gga gat gca gtg 865
Gly Ser Trp Ser Leu Pro Val Thr Val Asp Leu Pro Gly Asp Ala Val
270 275 280 285

gca ctt gga ctg caa tgc ttt acc ttg gac ctg aag aat gtt acc tgt 913
Ala Leu Gly Leu Gln Cys Phe Thr Leu Asp Leu Lys Asn Val Thr Cys
290 295 300

caa tgg cag caa cag gac cat gct agc tcc caa ggc ttc ttc tac cac 961
Gln Trp Gln Gln Gln Asp His Ala Ser Ser Gln Gly Phe Phe Tyr His
305 310 315

agc agg gca cgg tgc tgc ccc aga gac agg tac ccc atc tgg gag aac 1009
Ser Arg Ala Arg Cys Cys Pro Arg Asp Arg Tyr Pro Ile Trp Glu Asn
320 325 330

tgc gaa gag gaa gag aaa aca aat cca gga cta cag acc cca cag ttc 1057
Cys Glu Glu Glu Glu Lys Thr Asn Pro Gly Leu Gln Thr Pro Gln Phe
335 340 345

tct cgc tgc cac ttc aag tca cga aat gac agc att att cac atc ctt 1105
Ser Arg Cys His Phe Lys Ser Arg Asn Asp Ser Ile Ile His Ile Leu
350 355 360 365

gtg gag gtg acc aca gcc ccg ggt act gtt cac agc tac ctg ggc tcc 1153
Val Glu Val Thr Thr Ala Pro Gly Thr Val His Ser Tyr Leu Gly Ser
370 375 380

cct ttc tgg atc cac cag gct gtg cgc ctc ccc acc cca aac ttg cac 1201
Pro Phe Trp Ile His Gln Ala Val Arg Leu Pro Thr Pro Asn Leu His
385 390 395

tgg agg gag atc tcc agt ggg cat ctg gaa ttg gag tgg cag cac cca 1249
Trp Arg Glu Ile Ser Ser Gly His Leu Glu Leu Glu Trp Gln His Pro
400 405 410

tcg tcc tgg gca gcc caa gag acc tgt tat caa ctc cga tac aca gga 1297
Ser Ser Trp Ala Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly
415 420 425

gaa ggc cat cag gac tgg aag gtg ctg gag ccg cct ctc ggg gcc cga 1345
Glu Gly His Gln Asp Trp Lys Val Leu Glu Pro Pro Leu Gly Ala Arg
430 435 440 445

gga ggg acc ctg gag ctg cgc ccg cga tct cgc tac cgt tta cag ctg 1393
Gly Gly Thr Leu Glu Leu Arg Pro Arg Ser Arg Tyr Arg Leu Gln Leu
450 455 460

cgc gcc agg ctc aac ggc ccc acc tac caa ggt ccc tgg agc tcg tgg 1441
Arg Ala Arg Leu Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ser Trp
465 470 475

tcg gac cca act agg gtg gag acc gcc acc gag acc gcc tgg atc tcc 1489
Ser Asp Pro Thr Arg Val Glu Thr Ala Thr Glu Thr Ala Trp Ile Ser
480 485 490

ttg gtg acc gct ctg cat cta gtg ctg ggc ctc agc gcc gtc ctg ggc 1537
Leu Val Thr Ala Leu His Leu Val Leu Gly Leu Ser Ala Val Leu Gly
495 500 505

ctg ctg ctg ctg agg tgg cag ttt cct gca cac tac agg aga ctg agg 1585
Leu Leu Leu Leu Arg Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg
510 515 520 525

cat gcc ctg tgg ccc tca ctt cca gac ctg cac cgg gtc cta ggc cag 1633
His Ala Leu Trp Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln
530 535 540

tac ctt agg gac act gca gcc ctg agc ccg ccc aag gcc aca gtc tca 1681
Tyr Leu Arg Asp Thr Ala Ala Leu Ser Pro Pro Lys Ala Thr Val Ser
545 550 555

gat acc tgt gaa gaa gtg gaa ccc agc ctc ctt gaa atc ctc ccc aag 1729
Asp Thr Cys Glu Glu Val Glu Pro Ser Leu Leu Glu Ile Leu Pro Lys
560 565 570

tcc tca gag agg act cct ttg ccc ctg tgt tcc tcc cag gcc cag atg 1777
Ser Ser Glu Arg Thr Pro Leu Pro Leu Cys Ser Ser Gln Ala Gln Met
575 580 585

gac tac cga aga ttg cag cct tct tgc ctg ggg acc atg ccc ctg tct 1825
Asp Tyr Arg Arg Leu Gln Pro Ser Cys Leu Gly Thr Met Pro Leu Ser
590 595 600 605

gtg tgc cca ccc atg gct gag tca ggg tcc tgc tgt acc acc cac att 1873
Val Cys Pro Pro Met Ala Glu Ser Gly Ser Cys Cys Thr Thr His Ile
610 615 620

gcc aac cat tcc tac cta cca cta agc tat tgg cag cag cct tga 1918
Ala Asn His Ser Tyr Leu Pro Leu Ser Tyr Trp Gln Gln Pro
625 630 635

gtcgac 1924

<210> 248
<211> 635
<212> PRT
<213> Homo sapiens

<400> 248

Met Pro Ser Trp Ala Leu Phe Met Val Thr Ser Cys Leu Leu Leu Ala
1 5 10 15

Pro Gln Asn Leu Ala Gln Val Ser Ser Gln Asp Val Ser Leu Leu Ala
20 25 30

Ser Asp Ser Glu Pro Leu Lys Cys Phe Ser Arg Thr Phe Glu Asp Leu
35 40 45

Thr Cys Phe Trp Asp Glu Glu Glu Ala Ala Pro Ser Gly Thr Tyr Gln
50 55 60

Leu Leu Tyr Ala Tyr Pro Arg Glu Lys Pro Arg Ala Cys Pro Leu Ser

65 70 75 80

Ser Gln Ser Met Pro His Phe Gly Thr Arg Tyr Val Cys Gln Phe Pro
85 90 95

Asp Gln Glu Glu Val Pro Leu Phe Phe Pro Leu His Leu Trp Val Lys
100 105 110

Asn Val Phe Leu Asn Gln Thr Arg Thr Gln Arg Val Leu Phe Val Asp
115 120 125

Ser Val Gly Leu Pro Ala Pro Pro Ser Ile Ile Lys Ala Met Gly Gly
130 135 140

Ser Gln Pro Gly Glu Leu Gln Ile Ser Trp Glu Glu Pro Ala Pro Glu
145 150 155 160

Ile Ser Asp Phe Leu Arg Tyr Glu Leu Arg Tyr Gly Pro Arg Asp Pro
165 170 175

Lys Asn Ser Thr Gly Pro Thr Val Ile Gln Leu Ile Ala Thr Glu Thr
180 185 190

Cys Cys Pro Ala Leu Gln Arg Pro His Ser Ala Ser Ala Leu Asp Gln
195 200 205

Ser Pro Cys Ala Gln Pro Thr Met Pro Trp Gln Asp Gly Pro Lys Gln

210 215 220

Thr Ser Pro Ser Arg Glu Ala Ser Ala Leu Thr Ala Glu Gly Gly Ser
225 230 235 240

Cys Leu Ile Ser Gly Leu Gln Pro Gly Asn Ser Tyr Trp Leu Gln Leu
245 250 255

Arg Ser Glu Pro Asp Gly Ile Ser Leu Gly Gly Ser Trp Gly Ser Trp
260 265 270

Ser Leu Pro Val Thr Val Asp Leu Pro Gly Asp Ala Val Ala Leu Gly
275 280 285

Leu Gln Cys Phe Thr Leu Asp Leu Lys Asn Val Thr Cys Gln Trp Gln
290 295 300

Gln Gln Asp His Ala Ser Ser Gln Gly Phe Phe Tyr His Ser Arg Ala
305 310 315 320

Arg Cys Cys Pro Arg Asp Arg Tyr Pro Ile Trp Glu Asn Cys Glu Glu
325 330 335

Glu Glu Lys Thr Asn Pro Gly Leu Gln Thr Pro Gln Phe Ser Arg Cys
340 345 350

His Phe Lys Ser Arg Asn Asp Ser Ile Ile His Ile Leu Val Glu Val

355 360 365

Thr Thr Ala Pro Gly Thr Val His Ser Tyr Leu Gly Ser Pro Phe Trp
370 375 380

Ile His Gln Ala Val Arg Leu Pro Thr Pro Asn Leu His Trp Arg Glu
385 390 395 400

Ile Ser Ser Gly His Leu Glu Leu Glu Trp Gln His Pro Ser Ser Trp
405 410 415

Ala Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly Glu Gly His
420 425 430

Gln Asp Trp Lys Val Leu Glu Pro Pro Leu Gly Ala Arg Gly Gly Thr
435 440 445

Leu Glu Leu Arg Pro Arg Ser Arg Tyr Arg Leu Gln Leu Arg Ala Arg
450 455 460

Leu Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ser Trp Ser Asp Pro
465 470 475 480

Thr Arg Val Glu Thr Ala Thr Glu Thr Ala Trp Ile Ser Leu Val Thr
485 490 495

Ala Leu His Leu Val Leu Gly Leu Ser Ala Val Leu Gly Leu Leu Leu

500 505 510

Leu Arg Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu
515 520 525

Trp Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg
530 535 540

Asp Thr Ala Ala Leu Ser Pro Pro Lys Ala Thr Val Ser Asp Thr Cys
545 550 555 560

Glu Glu Val Glu Pro Ser Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu
565 570 575

Arg Thr Pro Leu Pro Leu Cys Ser Ser Gln Ala Gln Met Asp Tyr Arg
580 585 590

Arg Leu Gln Pro Ser Cys Leu Gly Thr Met Pro Leu Ser Val Cys Pro
595 600 605

Pro Met Ala Glu Ser Gly Ser Cys Cys Thr Thr His Ile Ala Asn His
610 615 620

Ser Tyr Leu Pro Leu Ser Tyr Trp Gln Gln Pro
625 630 635

<210> 249

<211> 1924
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (11)..(1918)
<223>

<400> 249
gaattccacc atg ccc tcc tgg gcc ctc ttc atg gtc acc tcc tgc ctc 49
Met Pro Ser Trp Ala Leu Phe Met Val Thr Ser Cys Leu
1 5 10

ctc ctg gcc cct caa aac ctg gcc caa gtc agc agc caa gat gtc tcc 97
Leu Leu Ala Pro Gln Asn Leu Ala Gln Val Ser Ser Gln Asp Val Ser
15 20 25

ttg ctg gca tca gac tca gag ccc ctg aag tgt ttc tcc cga aca ttt 145
Leu Leu Ala Ser Asp Ser Glu Pro Leu Lys Cys Phe Ser Arg Thr Phe
30 35 40 45

gag gac ctc act tgc ttc tgg gat gag gaa gag gca gcg ccc agt ggg 193
Glu Asp Leu Thr Cys Phe Trp Asp Glu Glu Glu Ala Ala Pro Ser Gly
50 55 60

aca tac cag ctg ctg tat gcc tac ccg cgg gag aag ccc cgt gct tgc 241
Thr Tyr Gln Leu Leu Tyr Ala Tyr Pro Arg Glu Lys Pro Arg Ala Cys
65 70 75

ccc ctg agt tcc cag agc atg ccc cac ttt gga acc cga tac gtg tgc 289
Pro Leu Ser Ser Gln Ser Met Pro His Phe Gly Thr Arg Tyr Val Cys
80 85 90

cag ttt cca gac cag gag gaa gtg cgt ctc ttc ttt ccg ctg cac ctc 337
Gln Phe Pro Asp Gln Glu Glu Val Arg Leu Phe Phe Pro Leu His Leu

95 100 105

tgg gtg aag aat gtg ttc cta aac cag act cgg act cag cga gtc ctc 385
Trp Val Lys Asn Val Phe Leu Asn Gln Thr Arg Thr Gln Arg Val Leu
110 115 120 125

ttt gtg gac agt gta ggc ctg ccg gct ccc ccc agt atc atc aag gcc 433
Phe Val Asp Ser Val Gly Leu Pro Ala Pro Pro Ser Ile Ile Lys Ala
130 135 140

atg ggt ggg agc cag cca ggg gaa ctt cag atc agc tgg gag gag cca 481
Met Gly Gly Ser Gln Pro Gly Glu Leu Gln Ile Ser Trp Glu Glu Pro
145 150 155

gct cca gaa atc agt gat ttc ctg agg tac gaa ctc cgc tat ggc ccc 529
Ala Pro Glu Ile Ser Asp Phe Leu Arg Tyr Glu Leu Arg Tyr Gly Pro
160 165 170

aga gat ccc aag aac tcc act ggt ccc acg gtc ata cag ctg att gcc 577
Arg Asp Pro Lys Asn Ser Thr Gly Pro Thr Val Ile Gln Leu Ile Ala
175 180 185

aca gaa acc tgc tgc cct gct ctg cag aga cct cac tca gcc tct gct 625
Thr Glu Thr Cys Cys Pro Ala Leu Gln Arg Pro His Ser Ala Ser Ala
190 195 200 205

ctg gac cag tct cca tgt gct cag ccc aca atg ccc tgg caa gat gga 673
Leu Asp Gln Ser Pro Cys Ala Gln Pro Thr Met Pro Trp Gln Asp Gly
210 215 220

cca aag cag acc tcc cca agt aga gaa gct tca gct ctg aca gca gag 721
Pro Lys Gln Thr Ser Pro Ser Arg Glu Ala Ser Ala Leu Thr Ala Glu
225 230 235

ggt gga agc tgc ctc atc tca gga ctc cag cct ggc aac tcc tac tgg 769
Gly Gly Ser Cys Leu Ile Ser Gly Leu Gln Pro Gly Asn Ser Tyr Trp

240 245 250

ctg cag ctg tgc agc gaa cct gat ggg atc tcc ctc ggt ggc tcc tgg 817
Leu Gln Leu Cys Ser Glu Pro Asp Gly Ile Ser Leu Gly Gly Ser Trp
255 260 265

gga tcc tgg tcc ctc cct gtg act gtg gac ctg cct gga gat gca gtg 865
Gly Ser Trp Ser Leu Pro Val Thr Val Asp Leu Pro Gly Asp Ala Val
270 275 280 285

gca ctt gga ctg caa tgc ttt acc ttg gac ctg aag aat gtt acc tgt 913
Ala Leu Gly Leu Gln Cys Phe Thr Leu Asp Leu Lys Asn Val Thr Cys
290 295 300

caa tgg cag caa cag gac cat gct agc tcc caa ggc ttc ttc tac cac 961
Gln Trp Gln Gln Gln Asp His Ala Ser Ser Gln Gly Phe Phe Tyr His
305 310 315

agc agg gca cgg tgc tgc ccc aga gac agg tac ccc atc tgg gag aac 1009
Ser Arg Ala Arg Cys Cys Pro Arg Asp Arg Tyr Pro Ile Trp Glu Asn
320 325 330

tgc gaa gag gaa gag aaa aca aat cca gga cta cag acc cca cag ttc 1057
Cys Glu Glu Glu Glu Lys Thr Asn Pro Gly Leu Gln Thr Pro Gln Phe
335 340 345

tct cgc tgc cac ttc aag tca cga aat gac agc att att cac atc ctt 1105
Ser Arg Cys His Phe Lys Ser Arg Asn Asp Ser Ile Ile His Ile Leu
350 355 360 365

gtg gag gtg acc aca gcc ccg ggt act gtt cac agc tac ctg ggc tcc 1153
Val Glu Val Thr Thr Ala Pro Gly Thr Val His Ser Tyr Leu Gly Ser
370 375 380

cct ttc tgg atc cac cag gct gtg cgc ctc ccc acc cca aac ttg cac 1201
Pro Phe Trp Ile His Gln Ala Val Arg Leu Pro Thr Pro Asn Leu His

385 390 395

tgg agg gag atc tcc agt ggg cat ctg gaa ttg gag tgg cag cac cca 1249
Trp Arg Glu Ile Ser Ser Gly His Leu Glu Leu Glu Trp Gln His Pro
400 405 410

tcg tcc tgg gca gcc caa gag acc tgt tat caa ctc cga tac aca gga 1297
Ser Ser Trp Ala Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly
415 420 425

gaa ggc cat cag gac tgg aag gtg ctg gag ccg cct ctc ggg gcc cga 1345
Glu Gly His Gln Asp Trp Lys Val Leu Glu Pro Pro Leu Gly Ala Arg
430 435 440 445

gga ggg acc ctg gag ctg cgc ccg cga tct cgc tac cgt tta cag ctg 1393
Gly Gly Thr Leu Glu Leu Arg Pro Arg Ser Arg Tyr Arg Leu Gln Leu
450 455 460

cgc gcc agg ctc aac ggc ccc acc tac caa ggt ccc tgg agc tcg tgg 1441
Arg Ala Arg Leu Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ser Trp
465 470 475

tcg gac cca act agg gtg gag acc gcc acc gag acc gcc tgg atc tcc 1489
Ser Asp Pro Thr Arg Val Glu Thr Ala Thr Glu Thr Ala Trp Ile Ser
480 485 490

ttg gtg acc gct ctg cat cta gtg ctg ggc ctc agc gcc gtc ctg ggc 1537
Leu Val Thr Ala Leu His Leu Val Leu Gly Leu Ser Ala Val Leu Gly
495 500 505

ctg ctg ctg ctg agg tgg cag ttt cct gca cac tac agg aga ctg agg 1585
Leu Leu Leu Leu Arg Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg
510 515 520 525

cat gcc ctg tgg ccc tca ctt cca gac ctg cac cgg gtc cta ggc cag 1633
His Ala Leu Trp Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln

530 535 540

tac ctt agg gac act gca gcc ctg agc ccg ccc aag gcc aca gtc tca 1681
Tyr Leu Arg Asp Thr Ala Ala Leu Ser Pro Pro Lys Ala Thr Val Ser
545 550 555

gat acc tgt gaa gaa gtg gaa ccc agc ctc ctt gaa atc ctc ccc aag 1729
Asp Thr Cys Glu Glu Val Glu Pro Ser Leu Leu Glu Ile Leu Pro Lys
560 565 570

tcc tca gag agg act cct ttg ccc ctg tgt tcc tcc cag gcc cag atg 1777
Ser Ser Glu Arg Thr Pro Leu Pro Leu Cys Ser Ser Gln Ala Gln Met
575 580 585

gac tac cga aga ttg cag cct tct tgc ctg ggg acc atg ccc ctg tct 1825
Asp Tyr Arg Arg Leu Gln Pro Ser Cys Leu Gly Thr Met Pro Leu Ser
590 595 600 605

gtg tgc cca ccc atg gct gag tca ggg tcc tgc tgt acc acc cac att 1873
Val Cys Pro Pro Met Ala Glu Ser Gly Ser Cys Cys Thr Thr His Ile
610 615 620

gcc aac cat tcc tac cta cca cta agc tat tgg cag cag cct tga 1918
Ala Asn His Ser Tyr Leu Pro Leu Ser Tyr Trp Gln Gln Pro
625 630 635

gtcgac 1924

<210> 250
<211> 635
<212> PRT
<213> Homo sapiens

<400> 250

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20 25 30

Ser Asp Ser Glu Pro Leu Lys Cys Phe Ser Arg Thr Phe Glu Asp Leu
35 40 45

Thr Cys Phe Trp Asp Glu Glu Glu Ala Ala Pro Ser Gly Thr Tyr Gln
50 55 60

Leu Leu Tyr Ala Tyr Pro Arg Glu Lys Pro Arg Ala Cys Pro Leu Ser
65 70 75 80

Ser Gln Ser Met Pro His Phe Gly Thr Arg Tyr Val Cys Gln Phe Pro
85 90 95

Asp Gln Glu Glu Val Arg Leu Phe Phe Pro Leu His Leu Trp Val Lys
100 105 110

Asn Val Phe Leu Asn Gln Thr Arg Thr Gln Arg Val Leu Phe Val Asp
115 120 125

Ser Val Gly Leu Pro Ala Pro Pro Ser Ile Ile Lys Ala Met Gly Gly
130 135 140

Ser Gln Pro Gly Glu Leu Gln Ile Ser Trp Glu Glu Pro Ala Pro Glu
145 150 155 160

Ile Ser Asp Phe Leu Arg Tyr Glu Leu Arg Tyr Gly Pro Arg Asp Pro
165 170 175

Lys Asn Ser Thr Gly Pro Thr Val Ile Gln Leu Ile Ala Thr Glu Thr
180 185 190

Cys Cys Pro Ala Leu Gln Arg Pro His Ser Ala Ser Ala Leu Asp Gln
195 200 205

Ser Pro Cys Ala Gln Pro Thr Met Pro Trp Gln Asp Gly Pro Lys Gln
210 215 220

Thr Ser Pro Ser Arg Glu Ala Ser Ala Leu Thr Ala Glu Gly Gly Ser
225 230 235 240

Cys Leu Ile Ser Gly Leu Gln Pro Gly Asn Ser Tyr Trp Leu Gln Leu
245 250 255

Cys Ser Glu Pro Asp Gly Ile Ser Leu Gly Gly Ser Trp Gly Ser Trp
260 265 270

Ser Leu Pro Val Thr Val Asp Leu Pro Gly Asp Ala Val Ala Leu Gly
275 280 285

Leu Gln Cys Phe Thr Leu Asp Leu Lys Asn Val Thr Cys Gln Trp Gln
290 295 300

Gln Gln Asp His Ala Ser Ser Gln Gly Phe Phe Tyr His Ser Arg Ala
305 310 315 320

Arg Cys Cys Pro Arg Asp Arg Tyr Pro Ile Trp Glu Asn Cys Glu Glu
325 330 335

Glu Glu Lys Thr Asn Pro Gly Leu Gln Thr Pro Gln Phe Ser Arg Cys
340 345 350

His Phe Lys Ser Arg Asn Asp Ser Ile Ile His Ile Leu Val Glu Val
355 360 365

Thr Thr Ala Pro Gly Thr Val His Ser Tyr Leu Gly Ser Pro Phe Trp
370 375 380

Ile His Gln Ala Val Arg Leu Pro Thr Pro Asn Leu His Trp Arg Glu
385 390 395 400

Ile Ser Ser Gly His Leu Glu Leu Glu Trp Gln His Pro Ser Ser Trp
405 410 415

Ala Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly Glu Gly His
420 425 430

Gln Asp Trp Lys Val Leu Glu Pro Pro Leu Gly Ala Arg Gly Gly Thr
435 440 445

Leu Glu Leu Arg Pro Arg Ser Arg Tyr Arg Leu Gln Leu Arg Ala Arg
450 455 460

Leu Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ser Trp Ser Asp Pro
465 470 475 480

Thr Arg Val Glu Thr Ala Thr Glu Thr Ala Trp Ile Ser Leu Val Thr
485 490 495

Ala Leu His Leu Val Leu Gly Leu Ser Ala Val Leu Gly Leu Leu Leu
500 505 510

Leu Arg Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu
515 520 525

Trp Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg
530 535 540

Asp Thr Ala Ala Leu Ser Pro Pro Lys Ala Thr Val Ser Asp Thr Cys
545 550 555 560

Glu Glu Val Glu Pro Ser Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu
565 570 575

Arg Thr Pro Leu Pro Leu Cys Ser Ser Gln Ala Gln Met Asp Tyr Arg
580 585 590

Arg Leu Gln Pro Ser Cys Leu Gly Thr Met Pro Leu Ser Val Cys Pro
595 600 605

Pro Met Ala Glu Ser Gly Ser Cys Cys Thr Thr His Ile Ala Asn His
610 615 620

Ser Tyr Leu Pro Leu Ser Tyr Trp Gln Gln Pro
625 630 635

<210> 251
<211> 1924
<212> DNA
<213> Homo sapiens

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<223>

<400> 251
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Met Pro Ser Trp Ala Leu Phe Met Val Thr Ser Cys Leu
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ctc ctg gcc cct caa aac ctg gcc caa gtc agc agc caa gat gtc tcc 97
Leu Leu Ala Pro Gln Asn Leu Ala Gln Val Ser Ser Gln Asp Val Ser
15 20 25

ttg ctg gca tca gac tca gag ccc ctg aag tgt ttc tcc cga aca ttt 145

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Leu Leu Ala Ser Asp Ser Glu Pro Leu Lys Cys Phe Ser Arg Thr Phe
30                  35                  40                  45

gag gac ctc act tgc ttc tgg gat gag gaa gag gca gcg ccc agt ggg      193
Glu Asp Leu Thr Cys Phe Trp Asp Glu Glu Glu Ala Ala Pro Ser Gly
                50                  55                  60

aca tac cag ctg ctg tat gcc tac ccg cgg gag aag ccc cgt gct tgc      241
Thr Tyr Gln Leu Leu Tyr Ala Tyr Pro Arg Glu Lys Pro Arg Ala Cys
            65                  70                  75

ccc ctg agt tcc cag agc atg ccc cac ttt gga acc cga tac gtg tgc      289
Pro Leu Ser Ser Gln Ser Met Pro His Phe Gly Thr Arg Tyr Val Cys
        80                  85                  90

cag ttt cca gac cag gag gaa gtg cgt ctc ttc ttt ccg ctg cac ctc      337
Gln Phe Pro Asp Gln Glu Glu Val Arg Leu Phe Phe Pro Leu His Leu
    95                  100                 105

tgg gtg aag aat gtg ttc cta aac cag act cgg act cag cga gtc ctc      385
Trp Val Lys Asn Val Phe Leu Asn Gln Thr Arg Thr Gln Arg Val Leu
110                 115                 120                 125

ttt gtg gac agt gta ggc ctg ccg gct ccc ccc agt atc atc aag gcc      433
Phe Val Asp Ser Val Gly Leu Pro Ala Pro Pro Ser Ile Ile Lys Ala
                130                 135                 140

atg ggt ggg agc cag cca ggg gaa ctt cag atc agc tgg gag gag cca      481
Met Gly Gly Ser Gln Pro Gly Glu Leu Gln Ile Ser Trp Glu Glu Pro
            145                 150                 155

gct cca gaa atc agt gat ttc ctg agg tac gaa ctc cgc tat ggc ccc      529
Ala Pro Glu Ile Ser Asp Phe Leu Arg Tyr Glu Leu Arg Tyr Gly Pro
        160                 165                 170

aga gat ccc aag aac tcc act ggt ccc acg gtc ata cag ctg att gcc      577
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Arg Asp Pro Lys Asn Ser Thr Gly Pro Thr Val Ile Gln Leu Ile Ala
175 180 185

aca gaa acc tgc tgc cct gct ctg cag aga cct cac tca gcc tct gct 625
Thr Glu Thr Cys Cys Pro Ala Leu Gln Arg Pro His Ser Ala Ser Ala
190 195 200 205

ctg gac cag tct cca tgt gct cag ccc aca atg ccc tgg caa gat gga 673
Leu Asp Gln Ser Pro Cys Ala Gln Pro Thr Met Pro Trp Gln Asp Gly
210 215 220

cca aag cag acc tcc cca agt aga gaa gct tca gct ctg aca gca gag 721
Pro Lys Gln Thr Ser Pro Ser Arg Glu Ala Ser Ala Leu Thr Ala Glu
225 230 235

ggt gga agc tgc ctc atc tca gga ctc cag cct ggc aac tcc tac tgg 769
Gly Gly Ser Cys Leu Ile Ser Gly Leu Gln Pro Gly Asn Ser Tyr Trp
240 245 250

ctg cag ctg cgc agc gaa cct gat ggg atc tcc ctc ggt ggc tcc tgg 817
Leu Gln Leu Arg Ser Glu Pro Asp Gly Ile Ser Leu Gly Gly Ser Trp
255 260 265

gga tcc tgg tcc ctc act gtg act gtg gac ctg cct gga gat gca gtg 865
Gly Ser Trp Ser Leu Thr Val Thr Val Asp Leu Pro Gly Asp Ala Val
270 275 280 285

gca ctt gga ctg caa tgc ttt acc ttg gac ctg aag aat gtt acc tgt 913
Ala Leu Gly Leu Gln Cys Phe Thr Leu Asp Leu Lys Asn Val Thr Cys
290 295 300

caa tgg cag caa cag gac cat gct agc tcc caa ggc ttc ttc tac cac 961
Gln Trp Gln Gln Gln Asp His Ala Ser Ser Gln Gly Phe Phe Tyr His
305 310 315

agc agg gca cgg tgc tgc ccc aga gac agg tac ccc atc tgg gag aac 1009

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Ser Arg Ala Arg Cys Cys Pro Arg Asp Arg Tyr Pro Ile Trp Glu Asn
        320                 325                 330

tgc gaa gag gaa gag aaa aca aat cca gga cta cag acc cca cag ttc     1057
Cys Glu Glu Glu Glu Lys Thr Asn Pro Gly Leu Gln Thr Pro Gln Phe
    335                 340                 345

tct cgc tgc cac ttc aag tca cga aat gac agc att att cac atc ctt     1105
Ser Arg Cys His Phe Lys Ser Arg Asn Asp Ser Ile Ile His Ile Leu
350                 355                 360                 365

gtg gag gtg acc aca gcc ccg ggt act gtt cac agc tac ctg ggc tcc     1153
Val Glu Val Thr Thr Ala Pro Gly Thr Val His Ser Tyr Leu Gly Ser
                370                 375                 380

cct ttc tgg atc cac cag gct gtg cgc ctc ccc acc cca aac ttg cac     1201
Pro Phe Trp Ile His Gln Ala Val Arg Leu Pro Thr Pro Asn Leu His
            385                 390                 395

tgg agg gag atc tcc agt ggg cat ctg gaa ttg gag tgg cag cac cca     1249
Trp Arg Glu Ile Ser Ser Gly His Leu Glu Leu Glu Trp Gln His Pro
        400                 405                 410

tcg tcc tgg gca gcc caa gag acc tgt tat caa ctc cga tac aca gga     1297
Ser Ser Trp Ala Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly
    415                 420                 425

gaa ggc cat cag gac tgg aag gtg ctg gag ccg cct ctc ggg gcc cga     1345
Glu Gly His Gln Asp Trp Lys Val Leu Glu Pro Pro Leu Gly Ala Arg
430                 435                 440                 445

gga ggg acc ctg gag ctg cgc ccg cga tct cgc tac cgt tta cag ctg     1393
Gly Gly Thr Leu Glu Leu Arg Pro Arg Ser Arg Tyr Arg Leu Gln Leu
                450                 455                 460

cgc gcc agg ctc aac ggc ccc acc tac caa ggt ccc tgg agc tcg tgg     1441
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Arg Ala Arg Leu Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ser Trp
            465                 470                 475

tcg gac cca act agg gtg gag acc gcc acc gag acc gcc tgg atc tcc     1489
Ser Asp Pro Thr Arg Val Glu Thr Ala Thr Glu Thr Ala Trp Ile Ser
        480                 485                 490

ttg gtg acc gct ctg cat cta gtg ctg ggc ctc agc gcc gtc ctg ggc     1537
Leu Val Thr Ala Leu His Leu Val Leu Gly Leu Ser Ala Val Leu Gly
    495                 500                 505

ctg ctg ctg ctg agg tgg cag ttt cct gca cac tac agg aga ctg agg     1585
Leu Leu Leu Leu Arg Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg
510                 515                 520                 525

cat gcc ctg tgg ccc tca ctt cca gac ctg cac cgg gtc cta ggc cag     1633
His Ala Leu Trp Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln
                530                 535                 540

tac ctt agg gac act gca gcc ctg agc ccg ccc aag gcc aca gtc tca     1681
Tyr Leu Arg Asp Thr Ala Ala Leu Ser Pro Pro Lys Ala Thr Val Ser
            545                 550                 555

gat acc tgt gaa gaa gtg gaa ccc agc ctc ctt gaa atc ctc ccc aag     1729
Asp Thr Cys Glu Glu Val Glu Pro Ser Leu Leu Glu Ile Leu Pro Lys
        560                 565                 570

tcc tca gag agg act cct ttg ccc ctg tgt tcc tcc cag gcc cag atg     1777
Ser Ser Glu Arg Thr Pro Leu Pro Leu Cys Ser Ser Gln Ala Gln Met
    575                 580                 585

gac tac cga aga ttg cag cct tct tgc ctg ggg acc atg ccc ctg tct     1825
Asp Tyr Arg Arg Leu Gln Pro Ser Cys Leu Gly Thr Met Pro Leu Ser
590                 595                 600                 605

gtg tgc cca ccc atg gct gag tca ggg tcc tgc tgt acc acc cac att     1873
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Val Cys Pro Pro Met Ala Glu Ser Gly Ser Cys Cys Thr Thr His Ile
610 615 620

gcc aac cat tcc tac cta cca cta agc tat tgg cag cag cct tga 1918
Ala Asn His Ser Tyr Leu Pro Leu Ser Tyr Trp Gln Gln Pro
625 630 635

gtcgac 1924

<210> 252
<211> 635
<212> PRT
<213> Homo sapiens

<400> 252

Met Pro Ser Trp Ala Leu Phe Met Val Thr Ser Cys Leu Leu Leu Ala
1 5 10 15

Pro Gln Asn Leu Ala Gln Val Ser Ser Gln Asp Val Ser Leu Leu Ala
20 25 30

Ser Asp Ser Glu Pro Leu Lys Cys Phe Ser Arg Thr Phe Glu Asp Leu
35 40 45

Thr Cys Phe Trp Asp Glu Glu Glu Ala Ala Pro Ser Gly Thr Tyr Gln
50 55 60

Leu Leu Tyr Ala Tyr Pro Arg Glu Lys Pro Arg Ala Cys Pro Leu Ser
65 70 75 80

Ser Gln Ser Met Pro His Phe Gly Thr Arg Tyr Val Cys Gln Phe Pro
85 90 95

Asp Gln Glu Glu Val Arg Leu Phe Phe Pro Leu His Leu Trp Val Lys
100 105 110

Asn Val Phe Leu Asn Gln Thr Arg Thr Gln Arg Val Leu Phe Val Asp
115 120 125

Ser Val Gly Leu Pro Ala Pro Pro Ser Ile Ile Lys Ala Met Gly Gly
130 135 140

Ser Gln Pro Gly Glu Leu Gln Ile Ser Trp Glu Glu Pro Ala Pro Glu
145 150 155 160

Ile Ser Asp Phe Leu Arg Tyr Glu Leu Arg Tyr Gly Pro Arg Asp Pro
165 170 175

Lys Asn Ser Thr Gly Pro Thr Val Ile Gln Leu Ile Ala Thr Glu Thr
180 185 190

Cys Cys Pro Ala Leu Gln Arg Pro His Ser Ala Ser Ala Leu Asp Gln
195 200 205

Ser Pro Cys Ala Gln Pro Thr Met Pro Trp Gln Asp Gly Pro Lys Gln
210 215 220

Thr Ser Pro Ser Arg Glu Ala Ser Ala Leu Thr Ala Glu Gly Gly Ser
225 230 235 240

Cys Leu Ile Ser Gly Leu Gln Pro Gly Asn Ser Tyr Trp Leu Gln Leu
245 250 255

Arg Ser Glu Pro Asp Gly Ile Ser Leu Gly Gly Ser Trp Gly Ser Trp
260 265 270

Ser Leu Thr Val Thr Val Asp Leu Pro Gly Asp Ala Val Ala Leu Gly
275 280 285

Leu Gln Cys Phe Thr Leu Asp Leu Lys Asn Val Thr Cys Gln Trp Gln
290 295 300

Gln Gln Asp His Ala Ser Ser Gln Gly Phe Phe Tyr His Ser Arg Ala
305 310 315 320

Arg Cys Cys Pro Arg Asp Arg Tyr Pro Ile Trp Glu Asn Cys Glu Glu
325 330 335

Glu Glu Lys Thr Asn Pro Gly Leu Gln Thr Pro Gln Phe Ser Arg Cys
340 345 350

His Phe Lys Ser Arg Asn Asp Ser Ile Ile His Ile Leu Val Glu Val
355 360 365

Thr Thr Ala Pro Gly Thr Val His Ser Tyr Leu Gly Ser Pro Phe Trp
370 375 380

Ile His Gln Ala Val Arg Leu Pro Thr Pro Asn Leu His Trp Arg Glu
385 390 395 400

Ile Ser Ser Gly His Leu Glu Leu Glu Trp Gln His Pro Ser Ser Trp
405 410 415

Ala Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly Glu Gly His
420 425 430

Gln Asp Trp Lys Val Leu Glu Pro Pro Leu Gly Ala Arg Gly Gly Thr
435 440 445

Leu Glu Leu Arg Pro Arg Ser Arg Tyr Arg Leu Gln Leu Arg Ala Arg
450 455 460

Leu Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ser Trp Ser Asp Pro
465 470 475 480

Thr Arg Val Glu Thr Ala Thr Glu Thr Ala Trp Ile Ser Leu Val Thr
485 490 495

Ala Leu His Leu Val Leu Gly Leu Ser Ala Val Leu Gly Leu Leu Leu
500 505 510

Leu Arg Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu
515 520 525

Trp Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg
530 535 540

Asp Thr Ala Ala Leu Ser Pro Pro Lys Ala Thr Val Ser Asp Thr Cys
545 550 555 560

Glu Glu Val Glu Pro Ser Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu
565 570 575

Arg Thr Pro Leu Pro Leu Cys Ser Ser Gln Ala Gln Met Asp Tyr Arg
580 585 590

Arg Leu Gln Pro Ser Cys Leu Gly Thr Met Pro Leu Ser Val Cys Pro
595 600 605

Pro Met Ala Glu Ser Gly Ser Cys Cys Thr Thr His Ile Ala Asn His
610 615 620

Ser Tyr Leu Pro Leu Ser Tyr Trp Gln Gln Pro
625 630 635

<210> 253
<211> 1572
<212> DNA

<213> Homo sapiens

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tgcaaggctt ctggatacac cttcaccaac tcctggatga actgggtgag gcagaggcct 180

ggaaagggtc ttgagtgggt tggacggatt tatcctggag atggagaaac tatctacaat 240

gggaaattca gggtcagagt cacgattacc gcggacgaat ccacgagcac agcctacatg 300

gagctgagca gcctgagatc tgaggacacg gccgtgtatt actgtgcgag aggctatgat 360

gattactcgt ttgcttactg gggccaggga accacggtca ccgtctcttc aggtggtggt 420

ggatccggag gtggtggatc gggtggtgga ggatcggata ttgtgatgac tcagtctgca 480

ctctccctgc ccgtcacccc tggagagccg gcctccatct cctgcaggtc tagtaagagt 540

ctcctgcata gtaatggcaa cacttacttg tattggtacc tgcagaagcc agggcagtct 600

ccacagctcc tgatctatcg gatgtccaac cttgcctcag gggtccctga caggttcagt 660

ggcagtggat caggcacagc ttttacactg aaaatcagca gagtggaggc tgaggatgtt 720

ggggtttatt actgcatgca acatatagaa tatcctttta cgttcggcca agggaccaaa 780

ctggaaatca aaggaggtgg tggatcgggt ggtggtggtt cgggaggcgg tggatcgcag 840

gtgcagctgg tgcagtctgg acctgaggtg aagaagcctg gggcctcagt gaaggtctcc 900

tgcaaggctt ctggatacac cttcaccaac tcctggatga actgggtgag gcagaggcct 960

ggaaagggtc ttgagtgggt tggacggatt tatcctggag atggagaaac tatctacaat 1020

gggaaattca gggtcagagt cacgattacc gcggacgaat ccacgagcac agcctacatg 1080

gagctgagca gcctgagatc tgaggacacg gccgtgtatt actgtgcgag aggctatgat 1140

gattactcgt ttgcttactg gggccaggga accacggtca ccgtctcttc aggtggtggt 1200

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ctcctgcata gtaatggcaa cacttacttg tattggtacc tgcagaagcc agggcagtct 1380

ccacagctcc tgatctatcg gatgtccaac cttgcctcag gggtccctga caggttcagt 1440

ggcagtggat caggcacagc ttttacactg aaaatcagca gagtggaggc tgaggatgtt 1500

ggggtttatt actgcatgca acatatagaa tatcctttta cgttcggcca agggaccaaa 1560

ctggaaatca aa 1572

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<211> 524
<212> PRT
<213> Homo sapiens

<400> 254

Met Asp Trp Thr Trp Arg Phe Leu Phe Val Val Ala Ala Ala Thr Gly
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Val Gln Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Ser Trp Met Asn Trp Val Arg Gln Arg Pro Gly Lys Gly Leu
50 55 60

Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn
65 70 75 80

Gly Lys Phe Arg Val Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly
115 120 125

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
130 135 140

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser Ala
145 150 155 160

Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
165 170 175

Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr Trp
180 185 190

Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Arg Met
195 200 205

Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
210 215 220

Gly Thr Ala Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
225 230 235 240

Gly Val Tyr Tyr Cys Met Gln His Ile Glu Tyr Pro Phe Thr Phe Gly
245 250 255

Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly
260 265 270

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Pro
275 280 285

Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser
290 295 300

Gly Tyr Thr Phe Thr Asn Ser Trp Met Asn Trp Val Arg Gln Arg Pro
305 310 315 320

Gly Lys Gly Leu Glu Trp Val Gly Arg Ile Tyr Pro Gly Asp Gly Glu
325 330 335

Thr Ile Tyr Asn Gly Lys Phe Arg Val Arg Val Thr Ile Thr Ala Asp
340 345 350

Glu Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu
355 360 365

Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Tyr Asp Asp Tyr Ser Phe
370 375 380

Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
385 390 395 400

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met
405 410 415

Thr Gln Ser Ala Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser
420 425 430

Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr
435 440 445

Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu
450 455 460

Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser
465 470 475 480

Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Lys Ile Ser Arg Val Glu
485 490 495

Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His Ile Glu Tyr Pro
500 505 510

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
515 520

<210> 255
<211> 354
<212> DNA
<213> Homo sapiens

<400> 255
caggtgcagc tggtgcagtc tggacctgag gtgaagaagc ctggggcctc agtgaaggtc 60

tcctgcaagg cttctggata caccttcacc aactcctgga tgaactgggt gaggcagagg 120

cctggaaagg gtcttgagtg ggttggacgg atttatcctg gagatggaga aactatctac 180

aatgggaaat tcagggtcag agtcacgatt accgcggacg aatccacgag cacagcctac 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaggctat 300

gatgattact cgtttgctta ctggggccag ggaaccacgg tcaccgtctc ttca 354

<210> 256
<211> 118
<212> PRT
<213> Homo sapiens

<400> 256

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn Gly Lys Phe
50 55 60

Arg Val Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser

115

<210> 257
<211> 336
<212> DNA
<213> Homo sapiens

<400> 257
gatattgtga tgactcagtc tgcactctcc ctgcccgtca cccctggaga gccggcctcc 60

atctcctgca ggtctagtaa gagtctcctg catagtaatg gcaacactta cttgtattgg 120

tacctgcaga agccagggca gtctccacag ctcctgatct atcggatgtc caaccttgcc 180

tcaggggtcc ctgacaggtt cagtggcagt ggatcaggca cagcttttac actgaaaatc 240

agcagagtgg aggctgagga tgttggggtt tattactgca tgcaacatat agaatatcct 300

tttacgttcg gccaagggac caaactggaa atcaaa 336

<210> 258
<211> 112
<212> PRT
<213> Homo sapiens

<400> 258

Asp Ile Val Met Thr Gln Ser Ala Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Ile Glu Tyr Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 259
<211> 1572
<212> DNA
<213> Homo sapiens

<400> 259
atggactgga cctggaggtt cctctttgtg gtggcagcag ctacaggtgt ccagtcccag 60

gtgcagctgg tgcagtctgg acctgaggtg aagaagcctg gggcctcagt gaaggtctcc 120

tgcaaggctt ctggatacac cttcaccaac tcctggatga actggatcag gcagaggcct 180

ggaaagggtc ttgagtggat tggacggatt tatcctggag atggagaaac tatctacaat 240

gggaaattca gggtcagagt cacgattacc gcggacgaat ccacgagcac agcctacatg 300

gagctgagca gcctgagatc tgaggacacg gccgtgtatt actgtgcgag aggctatgat 360

gattactcgt ttgcttactg gggccaggga accctggtca ccgtctcttc aggtggtggt 420

ggatccggag gtggtggatc gggtggtgga ggatcggata ttgtgatgac tcagtctgca 480

ctctccctgc ccgtcacccc tggagagccg gcctccatct cctgcaggtc tagtaagagt 540

ctcctgcata gtaatggcaa cacttacttg tattggtacc tgcagaagcc agggcagtct 600

ccacagctcc tgatctatcg gatgtccaac cttgcctcag gggtccctga caggttcagt 660

ggcagtggat caggcacagc ttttacactg aaaatcagca gagtggaggc tgaggatgtt 720

ggggtttatt actgcatgca acatatagaa tatcctttta cgttcggcca agggaccaaa 780

ctggaaatca aaggaggtgg tggatcgggt ggtggtggtt cgggaggcgg tggatcgcag 840

gtgcagctgg tgcagtctgg acctgaggtg aagaagcctg gggcctcagt gaaggtctcc 900

tgcaaggctt ctggatacac cttcaccaac tcctggatga actggatcag gcagaggcct 960

ggaaagggtc ttgagtggat tggacggatt tatcctggag atggagaaac tatctacaat 1020

gggaaattca gggtcagagt cacgattacc gcggacgaat ccacgagcac agcctacatg 1080

gagctgagca gcctgagatc tgaggacacg gccgtgtatt actgtgcgag aggctatgat 1140

gattactcgt ttgcttactg gggccaggga accctggtca ccgtctcttc aggtggtggt 1200

ggatccggag gtggtggatc gggtggtgga ggatcggata ttgtgatgac tcagtctgca 1260

ctctccctgc ccgtcacccc tggagagccg gcctccatct cctgcaggtc tagtaagagt 1320

ctcctgcata gtaatggcaa cacttacttg tattggtacc tgcagaagcc agggcagtct 1380

ccacagctcc tgatctatcg gatgtccaac cttgcctcag gggtccctga caggttcagt 1440

ggcagtggat caggcacagc ttttacactg aaaatcagca gagtggaggc tgaggatgtt 1500

ggggtttatt actgcatgca acatatagaa tatcctttta cgttcggcca agggaccaaa 1560

ctggaaatca aa 1572

<210> 260
<211> 524
<212> PRT
<213> Homo sapiens

<400> 260

Met Asp Trp Thr Trp Arg Phe Leu Phe Val Val Ala Ala Ala Thr Gly
1 5 10 15

Val Gln Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Ser Trp Met Asn Trp Ile Arg Gln Arg Pro Gly Lys Gly Leu
50 55 60

Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn
65 70 75 80

Gly Lys Phe Arg Val Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly
115 120 125

Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
130 135 140

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser Ala
145 150 155 160

Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
165 170 175

Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr Trp
180 185 190

Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Arg Met
195 200 205

Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
210 215 220

Gly Thr Ala Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
225 230 235 240

Gly Val Tyr Tyr Cys Met Gln His Ile Glu Tyr Pro Phe Thr Phe Gly
245 250 255

Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly
260 265 270

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Pro
275 280 285

Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser
290 295 300

Gly Tyr Thr Phe Thr Asn Ser Trp Met Asn Trp Ile Arg Gln Arg Pro
305 310 315 320

Gly Lys Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Glu
325 330 335

Thr Ile Tyr Asn Gly Lys Phe Arg Val Arg Val Thr Ile Thr Ala Asp
340 345 350

Glu Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu
355 360 365

Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Tyr Asp Asp Tyr Ser Phe
370 375 380

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly
385 390 395 400

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met
405 410 415

Thr Gln Ser Ala Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser
420 425 430

Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr
435 440 445

Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu
450 455 460

Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser
465 470 475 480

Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Lys Ile Ser Arg Val Glu
485 490 495

Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His Ile Glu Tyr Pro
500 505 510

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
515 520

<210> 261
<211> 354
<212> DNA
<213> Homo sapiens

<400> 261
caggtgcagc tggtgcagtc tggacctgag gtgaagaagc ctggggcctc agtgaaggtc 60

tcctgcaagg cttctggata caccttcacc aactcctgga tgaactggat caggcagagg 120

cctggaaagg gtcttgagtg gattggacgg atttatcctg gagatggaga aactatctac 180

aatgggaaat tcagggtcag agtcacgatt accgcggacg aatccacgag cacagcctac 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaggctat 300

gatgattact cgtttgctta ctggggccag ggaaccctgg tcaccgtctc ttca 354

<210> 262
<211> 118
<212> PRT
<213> Homo sapiens

<400> 262

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Ser

20 25 30

Trp Met Asn Trp Ile Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn Gly Lys Phe
50 55 60

Arg Val Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 263
<211> 1572
<212> DNA
<213> Mus musculus

<400> 263
atggaatggc ctttgatctt tctcttcctc ctgtcaggaa ctgcaggtgt ccactcccag 60

gttcagctgc agcagtctgg acctgagctg gtgaagcctg gggcctcagt gaagatttcc 120

tgcaaggctt ctggctatgc attcactaac tcctggatga actgggtgaa gcagaggcct 180

ggaaagggtc ttgagtggat tggacggatt tatcctggag atggagaaac tatctacaat 240

gggaaattca gggtcaaggc cacactgact gcagacaaat cctccagcac agcctacatg 300

gatatcagca gcctgacatc tgaggactct gcggtctact tctgtgcaag aggctatgat 360

gattactcgt ttgcttactg gggccaaggg actctggtca ctgtctctgc aggtggtggt 420

ggttcgggtg gtggtggttc gggtggtggc ggatcggata ttgtgatgac tcaggctgca 480

ccctctatac ctgtcactcc tggagagtca gtatccatct cctgtaggtc tagtaagagt 540

ctcctgcata gtaatggcaa cacttacttg tattggttcc tgcagaggcc aggccagtct 600

cctcaactcc tgatatatcg gatgtccaac cttgcctcag gagtcccaga taggttcagt 660

ggcagtgggt caggaactgc tttcacactg agaatcagta gagtggaggc tgaggatgtg 720

ggtgtttatt actgtatgca acatatagaa tatcctttta cgttcggatc ggggaccaag 780

ctggaaataa aaggaggtgg tggatcgggt ggtggtggtt cgggaggcgg tggatcgcag 840

gttcagctgc agcagtctgg acctgagctg gtgaagcctg gggcctcagt gaagatttcc 900

tgcaaggctt ctggctatgc attcactaac tcctggatga actgggtgaa gcagaggcct 960

ggaaagggtc ttgagtggat tggacggatt tatcctggag atggagaaac tatctacaat 1020

gggaaattca gggtcaaggc cacactgact gcagacaaat cctccagcac agcctacatg 1080

gatatcagca gcctgacatc tgaggactct gcggtctact tctgtgcaag aggctatgat 1140

gattactcgt ttgcttactg gggccaaggg actctggtca ctgtctctgc aggtggtggt 1200

ggttcgggtg gtggtggttc gggtggtggc ggatcggata ttgtgatgac tcaggctgca 1260

ccctctatac ctgtcactcc tggagagtca gtatccatct cctgtaggtc tagtaagagt 1320

ctcctgcata gtaatggcaa cacttacttg tattggttcc tgcagaggcc aggccagtct 1380

cctcaactcc tgatatatcg gatgtccaac cttgcctcag gagtcccaga taggttcagt 1440

ggcagtgggt caggaactgc tttcacactg agaatcagta gagtggaggc tgaggatgtg 1500

ggtgtttatt actgtatgca acatatagaa tatcctttta cgttcggatc ggggaccaag 1560

ctggaaataa aa 1572

<210> 264
<211> 524
<212> PRT
<213> Mus musculus

<400> 264

Met Glu Trp Pro Leu Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
1 5 10 15

Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
20 25 30

Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe
35 40 45

Thr Asn Ser Trp Met Asn Trp Val Lys Gln Arg Pro Gly Lys Gly Leu

50 55 60

Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn
65 70 75 80

Gly Lys Phe Arg Val Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser
85 90 95

Thr Ala Tyr Met Asp Ile Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
100 105 110

Tyr Phe Cys Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly
115 120 125

Gln Gly Thr Leu Val Thr Val Ser Ala Gly Gly Gly Gly Ser Gly Gly
130 135 140

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ala Ala
145 150 155 160

Pro Ser Ile Pro Val Thr Pro Gly Glu Ser Val Ser Ile Ser Cys Arg
165 170 175

Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr Trp
180 185 190

Phe Leu Gln Arg Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Arg Met

195 200 205

Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
210 215 220

Gly Thr Ala Phe Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val
225 230 235 240

Gly Val Tyr Tyr Cys Met Gln His Ile Glu Tyr Pro Phe Thr Phe Gly
245 250 255

Ser Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly
260 265 270

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Gln Gln Ser Gly Pro
275 280 285

Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser
290 295 300

Gly Tyr Ala Phe Thr Asn Ser Trp Met Asn Trp Val Lys Gln Arg Pro
305 310 315 320

Gly Lys Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Glu
325 330 335

Thr Ile Tyr Asn Gly Lys Phe Arg Val Lys Ala Thr Leu Thr Ala Asp

340 345 350

Lys Ser Ser Ser Thr Ala Tyr Met Asp Ile Ser Ser Leu Thr Ser Glu
355 360 365

Asp Ser Ala Val Tyr Phe Cys Ala Arg Gly Tyr Asp Asp Tyr Ser Phe
370 375 380

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Gly Gly Gly
385 390 395 400

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met
405 410 415

Thr Gln Ala Ala Pro Ser Ile Pro Val Thr Pro Gly Glu Ser Val Ser
420 425 430

Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr
435 440 445

Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser Pro Gln Leu Leu
450 455 460

Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser
465 470 475 480

Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile Ser Arg Val Glu

485 490 495

Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His Ile Glu Tyr Pro
500 505 510

Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
515 520

<210> 265
<211> 30
<212> PRT
<213> Homo sapiens

<400> 265

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
20 25 30

<210> 266
<211> 5
<212> PRT
<213> Homo sapiens

<400> 266

Asn Ser Trp Met Asn
1 5

<210> 267
<211> 14
<212> PRT
<213> Homo sapiens

<400> 267

Trp Val Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Val Gly
1 5 10

<210> 268
<211> 17
<212> PRT
<213> Homo sapiens

<400> 268

Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn Gly Lys Phe Arg
1 5 10 15

Val

<210> 269
<211> 32
<212> PRT
<213> Homo sapiens

<400> 269

Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr Met Glu
1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 270
<211> 9
<212> PRT
<213> Homo sapiens

<400> 270

Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr
1 5

<210> 271
<211> 11
<212> PRT
<213> Homo sapiens

<400> 271

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
1 5 10

<210> 272
<211> 23
<212> PRT
<213> Homo sapiens

<400> 272

Asp Ile Val Met Thr Gln Ser Ala Leu Ser Leu Pro Val Thr Pro Gly

1 5 10 15

Glu Pro Ala Ser Ile Ser Cys
20

<210> 273
<211> 16
<212> PRT
<213> Homo sapiens

<400> 273

Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
1 5 10 15

<210> 274
<211> 15
<212> PRT
<213> Homo sapiens

<400> 274

Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
1 5 10 15

<210> 275
<211> 7
<212> PRT
<213> Homo sapiens

<400> 275

Arg Met Ser Asn Leu Ala Ser
1 5

<210> 276
<211> 32
<212> PRT
<213> Homo sapiens

<400> 276

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr
1 5 10 15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
20 25 30

<210> 277
<211> 9
<212> PRT
<213> Homo sapiens

<400> 277

Met Gln His Ile Glu Tyr Pro Phe Thr
1 5

<210> 278
<211> 10
<212> PRT
<213> Homo sapiens

<400> 278

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 279
<211> 30
<212> PRT
<213> Homo sapiens

<400> 279

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
20 25 30

<210> 280
<211> 5
<212> PRT
<213> Homo sapiens

<400> 280

Asn Ser Trp Met Asn
1 5

<210> 281
<211> 14
<212> PRT
<213> Homo sapiens

<400> 281

Trp Ile Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile Gly
1 5 10

<210> 282
<211> 17
<212> PRT
<213> Homo sapiens

<400> 282

Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn Gly Lys Phe Arg
1 5 10 15

Val

<210> 283
<211> 32
<212> PRT
<213> Homo sapiens

<400> 283

Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr Met Glu
1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 284
<211> 9
<212> PRT
<213> Homo sapiens

<400> 284

Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr
1 5

<210> 285
<211> 11
<212> PRT
<213> Homo sapiens

<400> 285

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 286
<211> 1572
<212> DNA
<213> Homo sapiens

<400> 286
atggactgga cctggaggtt cctctttgtg gtggcagcag ctacaggtgt ccagtcccag 60

gtgcagctgg tgcagtctgg acctgaggtg aagaagcctg gggcctcagt gaaggtctcc 120

tgcaaggctt ctggatacac cttcaccaac tcctggatga actgggtgag gcagaggcct 180

ggaaagggtc ttgagtggat tggacggatt tatcctggag atggagaaac tatctacaat 240

gggaaattca gggtcagagt cacgattacc gcggacgaat ccacgagcac agcctacatg 300

caactgagca gcctgagatc tgaggacacg gccgtgtatt actgtgcgag aggctatgat 360

gattactcgt ttgcttactg gggccaggga accacggtca ccgtctcttc aggtggtggt 420

ggatccggag gtggtggatc gggtggtgga ggatcggata ttgtgatgac tcagtctcca 480

ctctccctgc ccgtcacccc tggagagccg gcctccatct cctgcaggtc tagtaagagt 540

ctcctgcata gtaatggcaa cacttacttg tattggttcc tgcagaagcc agggcagtct 600

ccacagctcc tgatctatcg gatgtccaac cttgcctcag gggtccctga caggttcagt 660

ggcagtggat caggcacaga ttttacactg aaaatcagca gagtggaggc tgaggatgtt 720

ggggtttatt actgcatgca acatatagaa tatcctttta cgttcggcca agggaccaaa 780

ctggaaatca aaggaggtgg tggatcgggt ggtggtggtt cgggaggcgg tggatcgcag 840

gtgcagctgg tgcagtctgg acctgaggtg aagaagcctg gggcctcagt gaaggtctcc 900

tgcaaggctt ctggatacac cttcaccaac tcctggatga actgggtgag gcagaggcct 960

ggaaagggtc ttgagtggat tggacggatt tatcctggag atggagaaac tatctacaat 1020

gggaaattca gggtcagagt cacgattacc gcggacgaat ccacgagcac agcctacatg 1080

caactgagca gcctgagatc tgaggacacg gccgtgtatt actgtgcgag aggctatgat 1140

gattactcgt ttgcttactg gggccaggga accacggtca ccgtctcttc aggtggtggt 1200

ggatccggag gtggtggatc gggtggtgga ggatcggata ttgtgatgac tcagtctcca 1260

ctctccctgc ccgtcacccc tggagagccg gcctccatct cctgcaggtc tagtaagagt 1320

ctcctgcata gtaatggcaa cacttacttg tattggttcc tgcagaagcc agggcagtct 1380

ccacagctcc tgatctatcg gatgtccaac cttgcctcag gggtccctga caggttcagt 1440

ggcagtggat caggcacaga ttttacactg aaaatcagca gagtggaggc tgaggatgtt 1500

ggggtttatt actgcatgca acatatagaa tatcctttta cgttcggcca agggaccaaa 1560

ctggaaatca aa 1572

<210> 287
<211> 524
<212> PRT
<213> Homo sapiens

<400> 287

Met Asp Trp Thr Trp Arg Phe Leu Phe Val Val Ala Ala Ala Thr Gly
1 5 10 15

Val Gln Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Ser Trp Met Asn Trp Val Arg Gln Arg Pro Gly Lys Gly Leu
50 55 60

Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn
65 70 75 80

Gly Lys Phe Arg Val Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser
85 90 95

Thr Ala Tyr Met Gln Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly
115 120 125

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
130 135 140

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser Pro
145 150 155 160

Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
165 170 175

Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr Trp
180 185 190

Phe Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Arg Met
195 200 205

Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
210 215 220

Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
225 230 235 240

Gly Val Tyr Tyr Cys Met Gln His Ile Glu Tyr Pro Phe Thr Phe Gly
245 250 255

Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly
260 265 270

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Pro
275 280 285

Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser
290 295 300

Gly Tyr Thr Phe Thr Asn Ser Trp Met Asn Trp Val Arg Gln Arg Pro
305 310 315 320

Gly Lys Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Glu
325 330 335

Thr Ile Tyr Asn Gly Lys Phe Arg Val Arg Val Thr Ile Thr Ala Asp
340 345 350

Glu Ser Thr Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Arg Ser Glu
355 360 365

Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Tyr Asp Asp Tyr Ser Phe
370 375 380

Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
385 390 395 400

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met
405 410 415

Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser
420 425 430

Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr
435 440 445

Tyr Leu Tyr Trp Phe Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu
450 455 460

Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser
465 470 475 480

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu
485 490 495

Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His Ile Glu Tyr Pro
500 505 510

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
515 520

<210> 288
<211> 354
<212> DNA
<213> Homo sapiens

<400> 288
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tcctgcaagg cttctggata caccttcacc aactcctgga tgaactgggt gaggcagagg 120

cctggaaagg gtcttgagtg gattggacgg atttatcctg gagatggaga aactatctac 180

aatgggaaat tcagggtcag agtcacgatt accgcggacg aatccacgag cacagcctac 240

atgcaactga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaggctat 300

gatgattact cgtttgctta ctggggccag ggaaccacgg tcaccgtctc ttca 354

<210> 289
<211> 118
<212> PRT
<213> Homo sapiens

<400> 289

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Ser
20 25 30

Trp Met Asn Trp Val Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn Gly Lys Phe
50 55 60

Arg Val Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

<210> 290
<211> 336
<212> DNA
<213> Homo sapiens

<400> 290
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atctcctgca ggtctagtaa gagtctcctg catagtaatg gcaacactta cttgtattgg 120

ttcctgcaga agccagggca gtctccacag ctcctgatct atcggatgtc caaccttgcc 180

tcaggggtcc ctgacaggtt cagtggcagt ggatcaggca cagattttac actgaaaatc 240

agcagagtgg aggctgagga tgttggggtt tattactgca tgcaacatat agaatatcct 300

tttacgttcg gccaagggac caaactggaa atcaaa 336

<210> 291
<211> 112
<212> PRT
<213> Homo sapiens

<400> 291

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
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Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Ile Glu Tyr Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 292
<211> 1572
<212> DNA
<213> Homo sapiens

<400> 292
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gtgcagctgg tgcagtctgg acctgaggtg aagaagcctg gggcctcagt gaaggtctcc 120

tgcaaggctt ctggatacac cttcaccaac tcctggatga actgggtgag gcagaggcct 180

ggaaagggtc ttgagtggat tggacggatt tatcctggag atggagaaac tatctacaat 240

gggaaattca gggtcagagt cacgattacc gcggacgaat ccacgagcac agcctacatg 300

gagctgagca gcctgagatc tgaggacacg gccgtgtatt actgtgcgag aggctatgat 360

gattactcgt ttgcttactg gggccaggga accacggtca ccgtctcttc aggtggtggt 420

ggatccggag gtggtggatc gggtggtgga ggatcggata ttgtgatgac tcagtctcca 480

ctctccctgc ccgtcacccc tggagagccg gcctccatct cctgcaggtc tagtaagagt 540

ctcctgcata gtaatggcaa cacttacttg tattggttcc agcagaagcc agggcaggct 600

ccacggctcc tgatctatcg gatgtccaac cttgcctcag gggtccctga caggttcagt 660

ggcagtggat caggcacagc ttttacactg aaaatcagca gagtggaggc tgaggatgtt 720

ggggtttatt actgcatgca acatatagaa tatcctttta cgttcggcca agggaccaaa 780

ctggaaatca aaggaggtgg tggatcgggt ggtggtggtt cgggaggcgg tggatcgcag 840

gtgcagctgg tgcagtctgg acctgaggtg aagaagcctg gggcctcagt gaaggtctcc 900

tgcaaggctt ctggatacac cttcaccaac tcctggatga actgggtgag gcagaggcct 960

ggaaagggtc ttgagtggat tggacggatt tatcctggag atggagaaac tatctacaat 1020

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ctcctgcata gtaatggcaa cacttacttg tattggttcc agcagaagcc agggcaggct 1380

ccacggctcc tgatctatcg gatgtccaac cttgcctcag gggtccctga caggttcagt 1440

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<210> 293

<211> 524
<212> PRT
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20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Ser Trp Met Asn Trp Val Arg Gln Arg Pro Gly Lys Gly Leu
50 55 60

Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn
65 70 75 80

Gly Lys Phe Arg Val Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly
115 120 125

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
130 135 140

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser Pro
145 150 155 160

Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
165 170 175

Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr Trp
180 185 190

Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Arg Met
195 200 205

Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
210 215 220

Gly Thr Ala Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
225 230 235 240

Gly Val Tyr Tyr Cys Met Gln His Ile Glu Tyr Pro Phe Thr Phe Gly
245 250 255

Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly
260 265 270

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Pro
275 280 285

Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser
290 295 300

Gly Tyr Thr Phe Thr Asn Ser Trp Met Asn Trp Val Arg Gln Arg Pro
305 310 315 320

Gly Lys Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly Asp Gly Glu
325 330 335

Thr Ile Tyr Asn Gly Lys Phe Arg Val Arg Val Thr Ile Thr Ala Asp
340 345 350

Glu Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu
355 360 365

Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Tyr Asp Asp Tyr Ser Phe
370 375 380

Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly
385 390 395 400

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met
405 410 415

Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser
420 425 430

Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr
435 440 445

Tyr Leu Tyr Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
450 455 460

Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser
465 470 475 480

Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Lys Ile Ser Arg Val Glu
485 490 495

Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His Ile Glu Tyr Pro
500 505 510

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
515 520

<210> 294
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<212> DNA
<213> Homo sapiens

<400> 294

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cctggaaagg gtcttgagtg gattggacgg atttatcctg gagatggaga aactatctac 180

aatgggaaat tcagggtcag agtcacgatt accgcggacg aatccacgag cacagcctac 240

atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaggctat 300

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<210> 295
<211> 118
<212> PRT
<213> Homo sapiens

<400> 295

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
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Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Ser
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Trp Met Asn Trp Val Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Asp Gly Glu Thr Ile Tyr Asn Gly Lys Phe
50 55 60

Arg Val Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Tyr Asp Asp Tyr Ser Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

<210> 296
<211> 336
<212> DNA
<213> Homo sapiens

<400> 296
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atctcctgca ggtctagtaa gagtctcctg catagtaatg gcaacactta cttgtattgg 120

ttccagcaga agccagggca ggctccacgg ctcctgatct atcggatgtc caaccttgcc 180

tcaggggtcc ctgacaggtt cagtggcagt ggatcaggca cagcttttac actgaaaatc 240

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tttacgttcg gccaagggac caaactggaa atcaaa 336

<210> 297

<211> 112
<212> PRT
<213> Homo sapiens

<400> 297

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Gln Gln Lys Pro Gly Gln Ala
35 40 45

Pro Arg Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95

Ile Glu Tyr Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 298
<211> 30

<212> PRT
<213> Homo sapiens

<400> 298

Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
20 25 30

<210> 299
<211> 14
<212> PRT
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<400> 299

Trp Val Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Ile Gly
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<210> 300
<211> 32
<212> PRT
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<400> 300

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1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg

20 25 30

<210> 301
<211> 11
<212> PRT
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<400> 301

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
1 5 10

<210> 302
<211> 23
<212> PRT
<213> Homo sapiens

<400> 302

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys
20

<210> 303
<211> 15
<212> PRT
<213> Homo sapiens

<400> 303

Trp Phe Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr
1 5 10 15

<210> 304
<211> 32
<212> PRT
<213> Homo sapiens

<400> 304

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
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<210> 305
<211> 10
<212> PRT
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<400> 305

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 306
<211> 32
<212> PRT
<213> Homo sapiens

<400> 306

Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr Met Glu
1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 307
<211> 15
<212> PRT
<213> Homo sapiens

<400> 307

Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
1 5 10 15

<210> 308
<211> 32
<212> PRT
<213> Homo sapiens

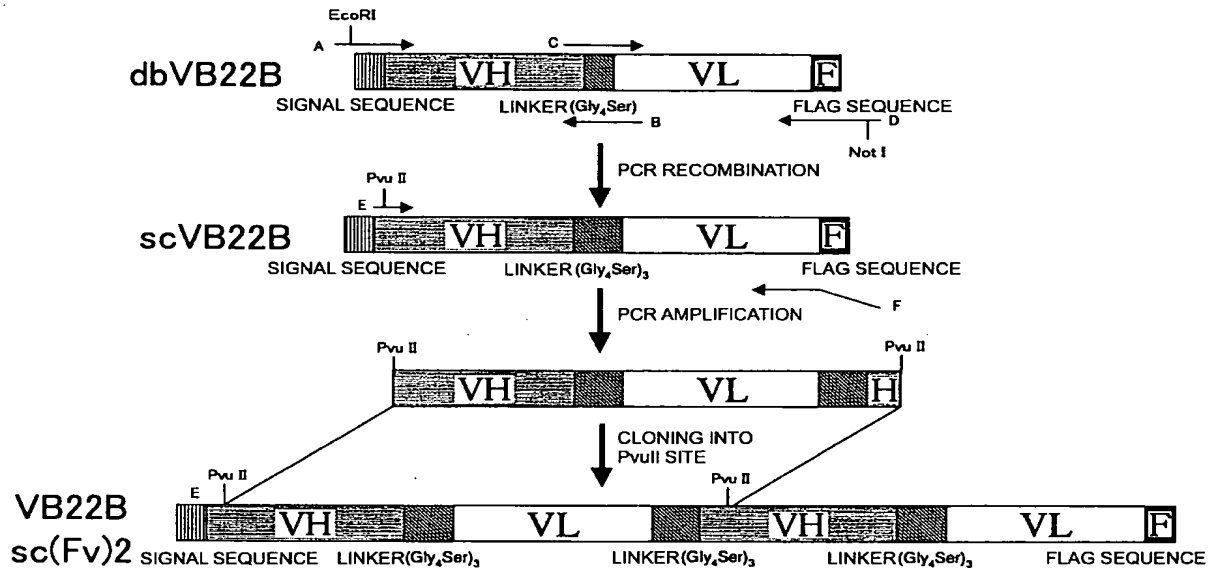
<400> 308

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr
1 5 10 15

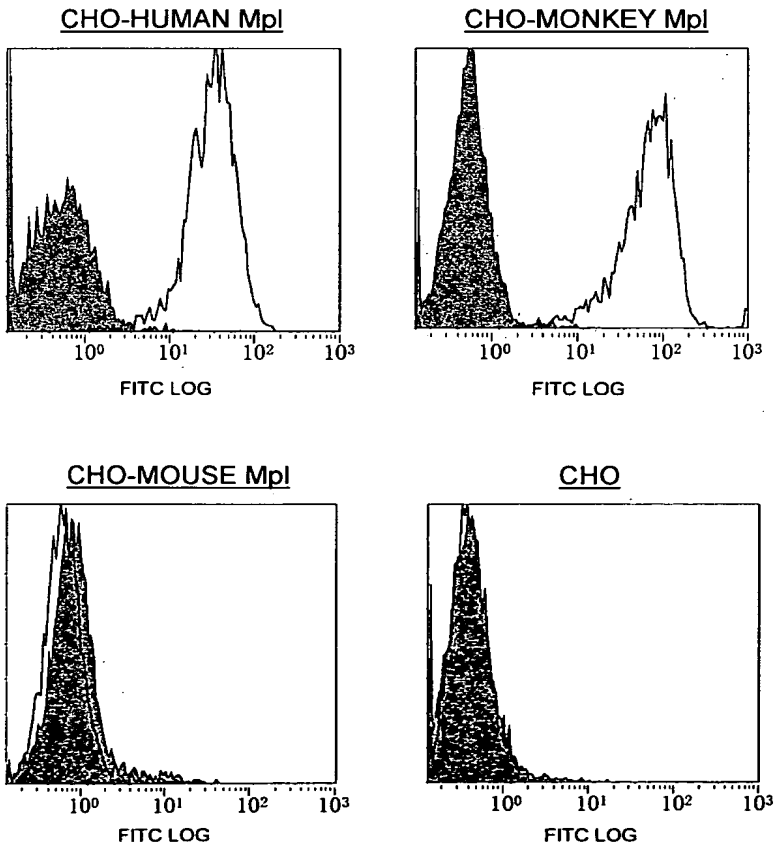
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
20 25 30

[Document Name] Drawings

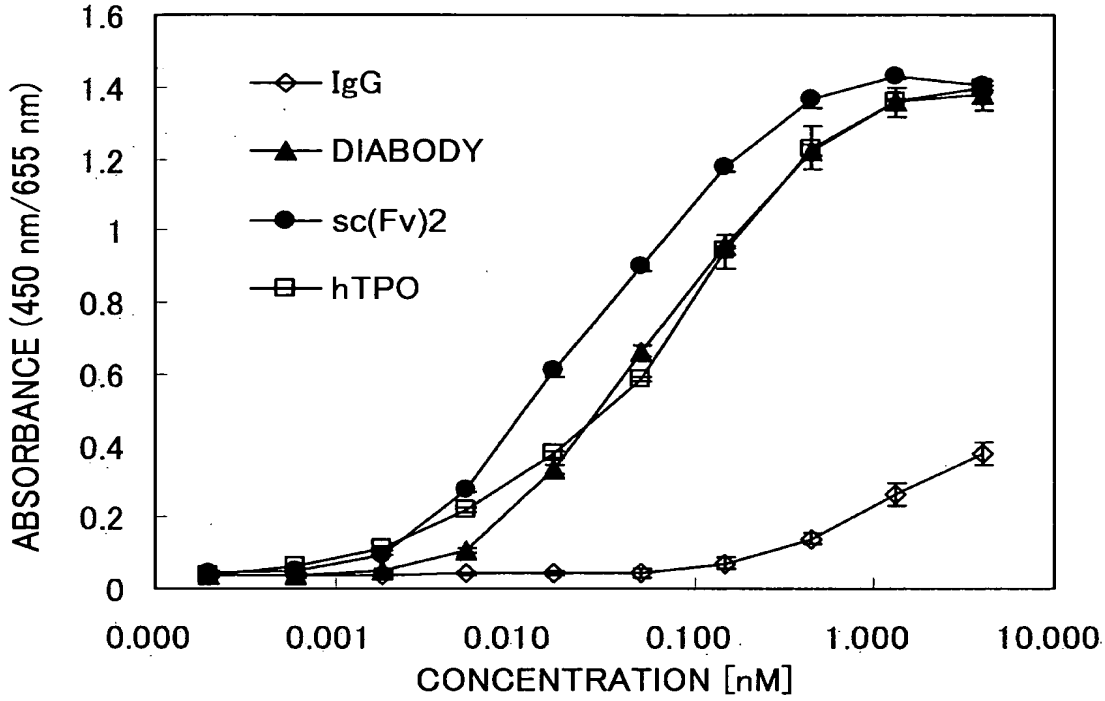
[Fig. 1]



[Fig. 2]



[Fig. 3]


ABSORBANCE (450 nm/655 nm)
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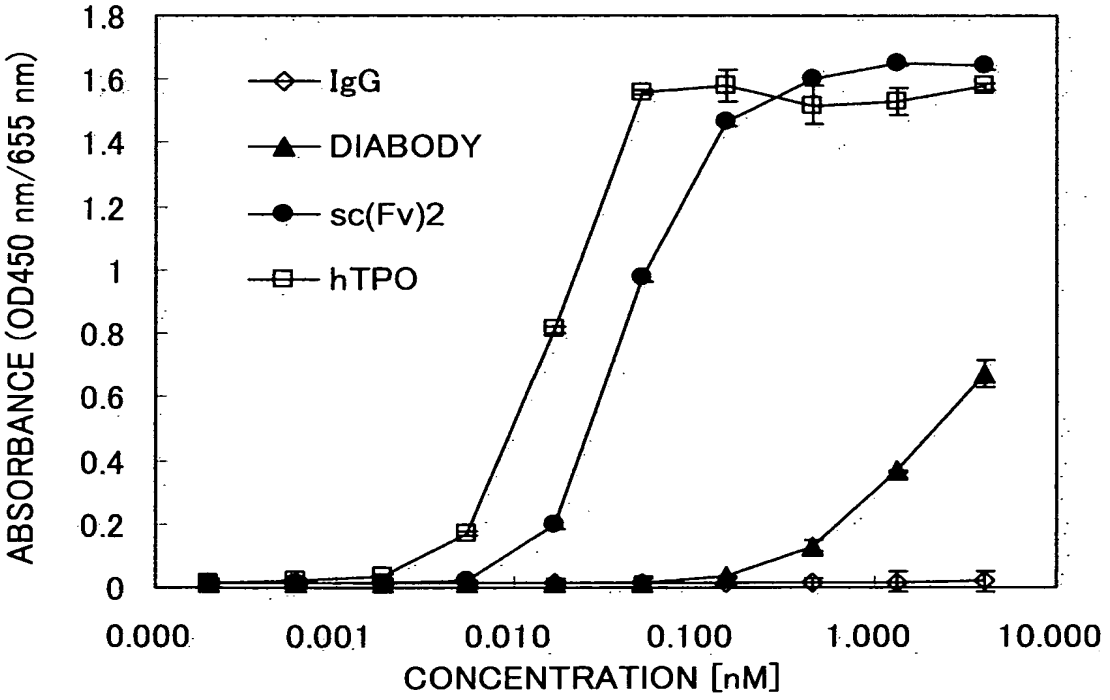
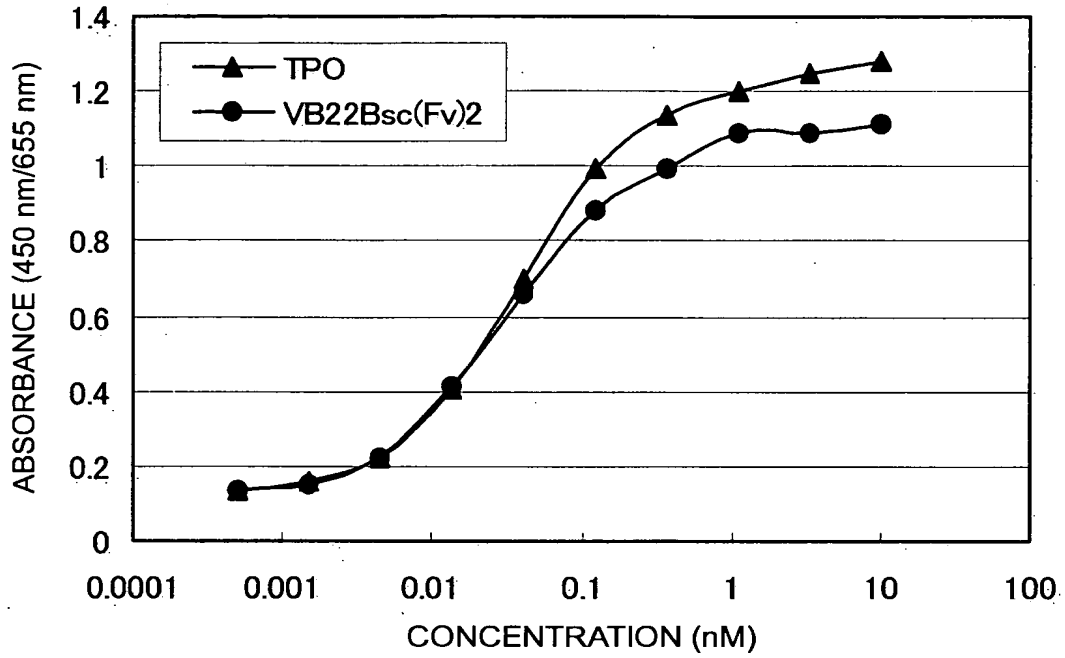
1.4
1.2
1
0.8
0.6
0.4
0.2
0
IgG
DIABODY
sc(Fv)2
hTPO
0.000
0.001
0.010
0.100
1.000
10.000
CONCENTRATION [nM]

[Fig. 4]


ABSORBANCE (OD450 nm/655 nm)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
IgG
DIABODY
sc(Fv)2
hTPO
0.000
0.001
0.010
0.100
1.000
10.000
CONCENTRATION [nM]

[Fig. 5]



[Fig. 6]

		CDR1		CDR2
VA7	QVQLQQSGPELVKPGASVKISCKASGYAFS	SSWMN	WVKQRPGKGLEWIG	RTYPGDGDTNYNGKFKG
VA130	QVQLQQSGPELVKPGASVKISCKASGYAFS	SSWMN	WVKQRPGKGLEWIG	RIYPGDGDTNYNGKFKG
VA259	QVQLQQSGPELVKPGASVKISCKASGYAFS	SSWMN	WVKQRPGKGLEWIG	RIYPGDGETNYNGKFKG
VB17B	QVQLQQSGPELVKPGASVKISCKASGYTFS	SSWMN	WVKQRPGKGLEWIG	RIYPGDGDTNYNGKFKG
VB12B	QVQLQQSGPELVKPGASVKISCKASGYAFS	RSWMN	WVKQRPGKGLEWIG	RIYPGDGDTNYNGKFKG
VB140	QVQLQQSGPELVKPGASVKISCRAFGYAFS	NSWMN	WVKQRPGKGLEWIG	RIYPGDGETNNNGKFKG
VB33	QVQLQQPGAELVKPGASVKLSCKASGYTFT	NYWVN	WVKQRPGRGLEWIG	RIHPSDSETHCNQKFKR
VB45B	QVQLQQSGPELVKPGASVKISCKASGYAFS	SSWMN	WVKQRPGKGLEWIG	RIYPGDGETNNNGKFKG
VB8B	QVQLQQSGPELVKPGASVKISCKASGYAFS	TSWMN	WVKQRPGKGLEWIG	RIYPGDGEANYNGKFKG
VB115	QVQLQQSGPELVKPGASVKISCKASGYAFS	SSWMN	WVKQRPGKGPEWIG	RIYPGDGETNYNGKFKG
VB14B	QVQLQQSGPELLNPGASVKISCKASGYAFS	RSWMN	WVKQRPGKGLEWIG	RIYPGDGETNYNGKFKG
VB22B	QVQLQQSGPELVKPGASVKISCKASGYAFT	NSWMN	WVKQRPGKGLEWIG	RIYPGDGETIYNGKFRV
VB16	QVQLQQPGTELVRPGASVKLSCKASGYTFT	DYWVN	WVKQRPGRGLEWIG	RIHPYDSETHYNQKFKN
VB157	QVQLQQPGAELVKPGASVKLSCKASGYTFT	DYWMN	WVKQRPGRGLEWIG	RIHPFDSETHCSQKFKN
VB4B	QVQLQQSGPELVKPGASVKISCKASGYAFT	NSWMN	WVRQRPGKGLEWIG	RIYPGDGETIYNGKFRV
VB51	QVQLQQSGPELVKPGASVKISCKASGYAFS	NSWMN	WVNQRPGKGLEWIG	RIYPGDGDTIYNGNFKG

		CDR3	
VA7	KATLTADKSSSTAYMQLSSLTSEDSAVYFCAR	GWILADGGYSFAY	WGQGTLVTVSA
VA130	KATLTADKSSSTAYIQLSSLTSEDSAVYFCAR	GYAD----YSFAY	WGQGTLVTVSA
VA259	KATLTADKSSNTAYMQLSSLTSEDSAVYFCAR	GFGD----YSFAY	WGQGTLVTVSA
VB17B	KATLTADKSSSTAYMQLSSLTSEDSAVYFCAS	GYAD----YSFAY	WGQGTLVTVSA
VB12B	KATLTADKSSSTAYMQLSSLTSEDSAVYFCAS	GYDD----YSFAY	WGQGTLVTVSA
VB140	KATLTADKSSSTAYMQLSSLTSEDSAVYFCAR	GYGD----YSFAY	WGQGTLVTVSA
VB33	KATLTVNKSSSTAYIQLHSLTSEDSAVYYCTS	GGW-------FAY	WGQGTLVTVSA
VB45B	KATLTADKSSTAYMQLSSLTSEDSAVYFCAR	GYGD----YSFAY	WGQGTLVTVSA
VB8B	KATLTADKSSSSAYMQLSSLTSEDSAVYFCAR	GYGD----YSFAY	WGQGTLVTVSA
VB115	KATLTADKSSSTVYMQLSSLTSEDSAVYFCAR	GYGD----YSFAY	WGQGTLVTVSA
VB14B	KATLTADKSSTAYMQFSSLTSEDSAVYFCAR	GDGD----YSFAY	WGQGTLVTVSA
VB22B	KATLTADKSSSTAYMDISSLTSEDSAVYFCAR	GYDD----YSFAY	WGQGTLVTVSA
VB16	KATLTVDKSSSTAYIQLSSLTSEDSAVYYCAS	GGW-------FAS	WGQGTLVTVSA
VB157	KATLTVDKSSNTAYIQFSSLTSEDSAVYYCSS	GGW-------FAY	WGQGTLVTVSA
VB4B	KATLTADKSSSTAYMEISSLTSEDSAVYFCAR	GYDD----YSFAY	WGQGTLVTVSA
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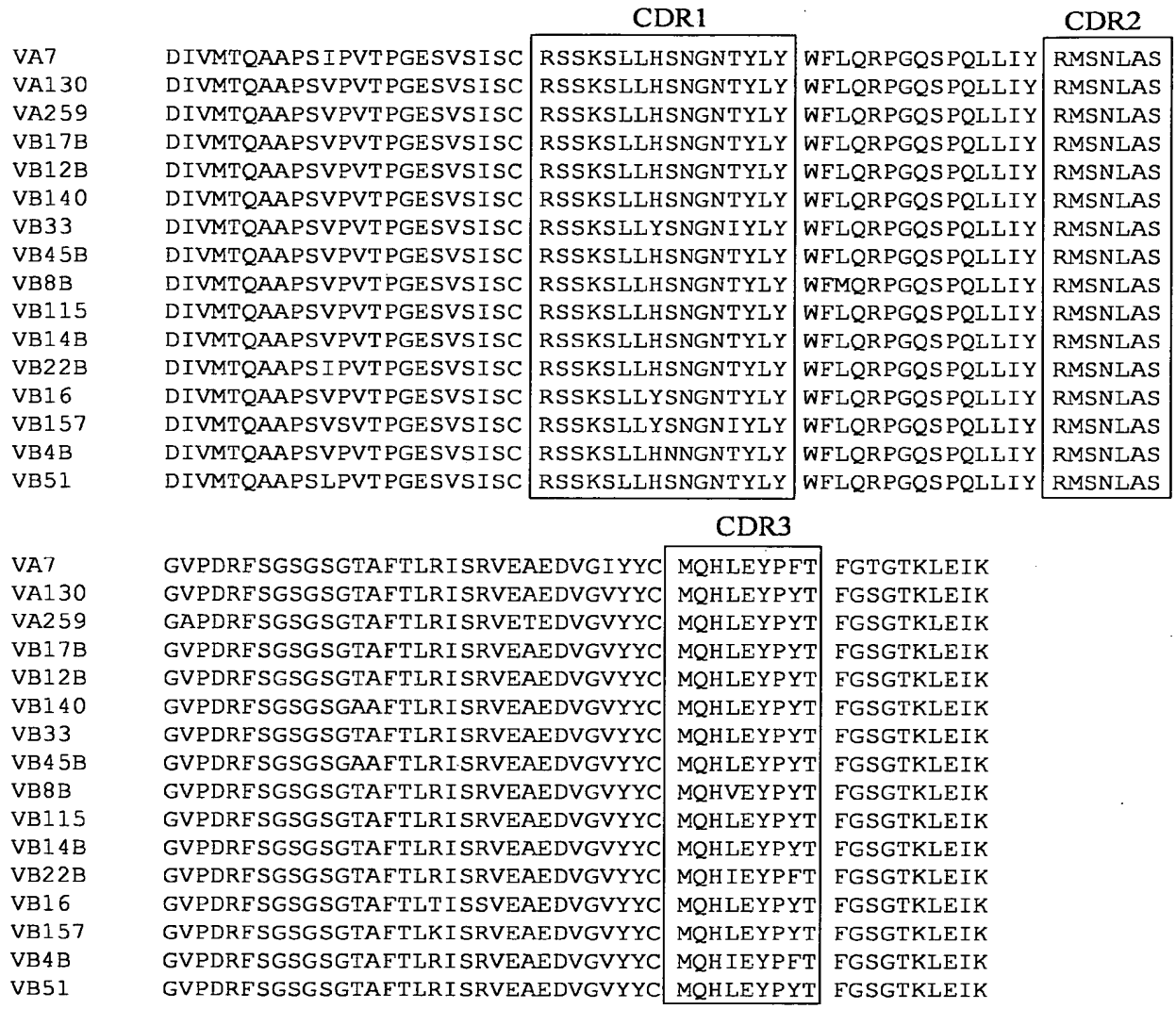
[Fig. 7]


CDR1
CDR2
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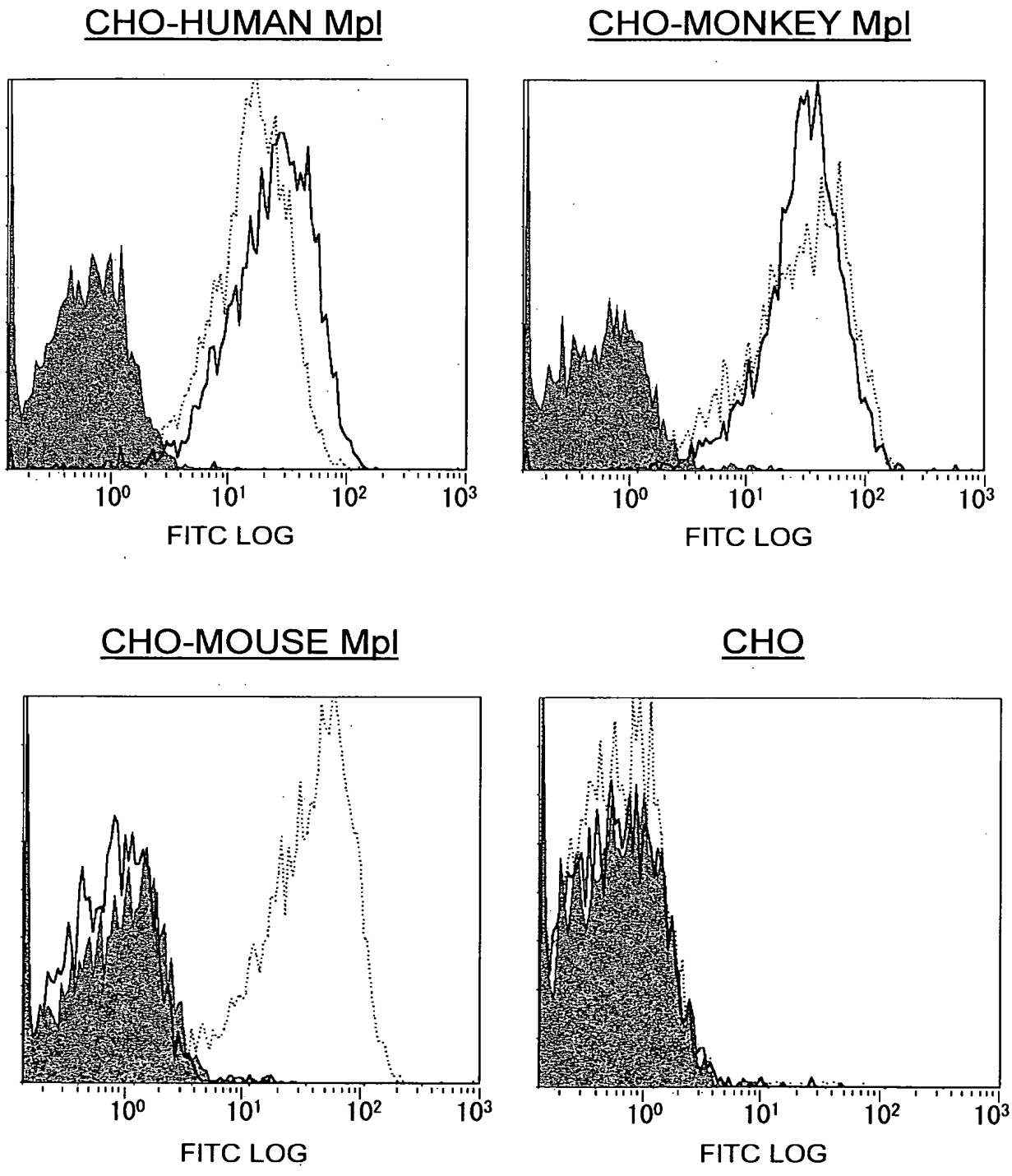
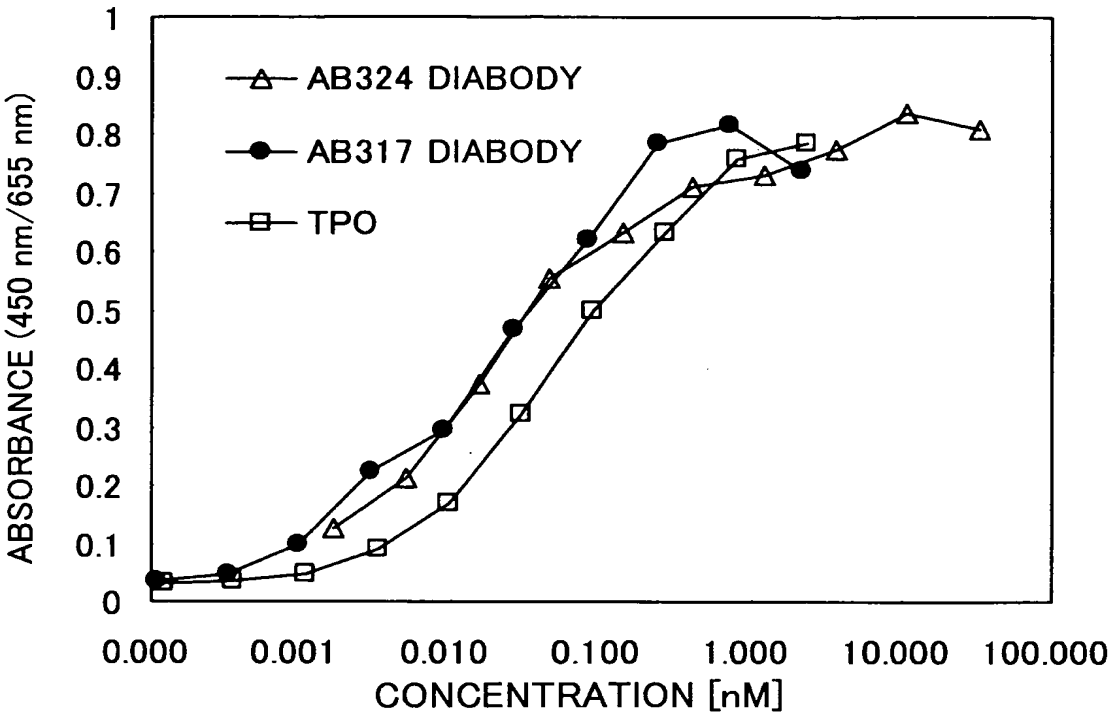
VA130 DIVMTQAAPSVPVTPGESVSISC RSSKSLLHSNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
VA259 DIVMTQAAPSVPVTPGESVSISC RSSKSLLHSNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
VB17B DIVMTQAAPSVPVTPGESVSISC RSSKSLLHSNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
VB12B DIVMTQAAPSVPVTPGESVSISC RSSKSLLHSNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
VB140 DIVMTQAAPSVPVTPGESVSISC RSSKSLLHSNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
VB33 DIVMTQAAPSVPVTPGESVSISC RSSKSLLYSNGNIYLY WFLQRPGQSPQLLIY RMSNLAS
VB45B DIVMTQAAPSVPVTPGESVSISC RSSKSLLHSNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
VB8B DIVMTQAAPSVPVTPGESVSISC RSSKSLLHSNGNTYLY WFMQRPGQSPQLLIY RMSNLAS
VB115 DIVMTQAAPSVPVTPGESVSISC RSSKSLLHSNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
VB14B DIVMTQAAPSVPVTPGESVSISC RSSKSLLHSNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
VB22B DIVMTQAAPSIPVTPGESVSISC RSSKSLLHSNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
VB16 DIVMTQAAPSVPVTPGESVSISC RSSKSLLYSNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
VB157 DIVMTQAAPSVSVTPGESVSISC RSSKSLLYSNGNIYLY WFLQRPGQSPQLLIY RMSNLAS
VB4B DIVMTQAAPSVPVTPGESVSISC RSSKSLLHNNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
VB51 DIVMTQAAPSLPVTPGESVSISC RSSKSLLHSNGNTYLY WFLQRPGQSPQLLIY RMSNLAS
CDR3
VA7 GVPDRFSGSGSGTAFTLRISRVEAEDVGIYYC MQHLEYPFT FGTGTKLEIK
VA130 GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC MQHLEYPYT FGSGTKLEIK
VA259 GAPDRFSGSGSGTAFTLRISRVETEDVGVYYC MQHLEYPYT FGSGTKLEIK
VB17B GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC MQHLEYPYT FGSGTKLEIK
VB12B GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC MQHLEYPYT FGSGTKLEIK
VB140 GVPDRFSGSGSGAAFTLRISRVEAEDVGVYYC MQHLEYPYT FGSGTKLEIK
VB33 GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC MQHLEYPYT FGSGTKLEIK
VB45B GVPDRFSGSGSGAAFTLRISRVEAEDVGVYYC MQHLEYPYT FGSGTKLEIK
VB8B GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC MQHVEYPYT FGSGTKLEIK
VB115 GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC MQHLEYPYT FGSGTKLEIK
VB14B GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC MQHLEYPYT FGSGTKLEIK
VB22B GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC MQHIEYPFT FGSGTKLEIK
VB16 GVPDRFSGSGSGTAFTLTISSVEAEDVGVYYC MQHLEYPYT FGSGTKLEIK
VB157 GVPDRFSGSGSGTAFTLKISRVEAEDVGVYYC MQHLEYPYT FGSGTKLEIK
VB4B GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC MQHIEYPFT FGSGTKLEIK
VB51 GVPDRFSGSGSGTAFTLRISRVEAEDVGVYYC MQHLEYPYT FGSGTKLEIK

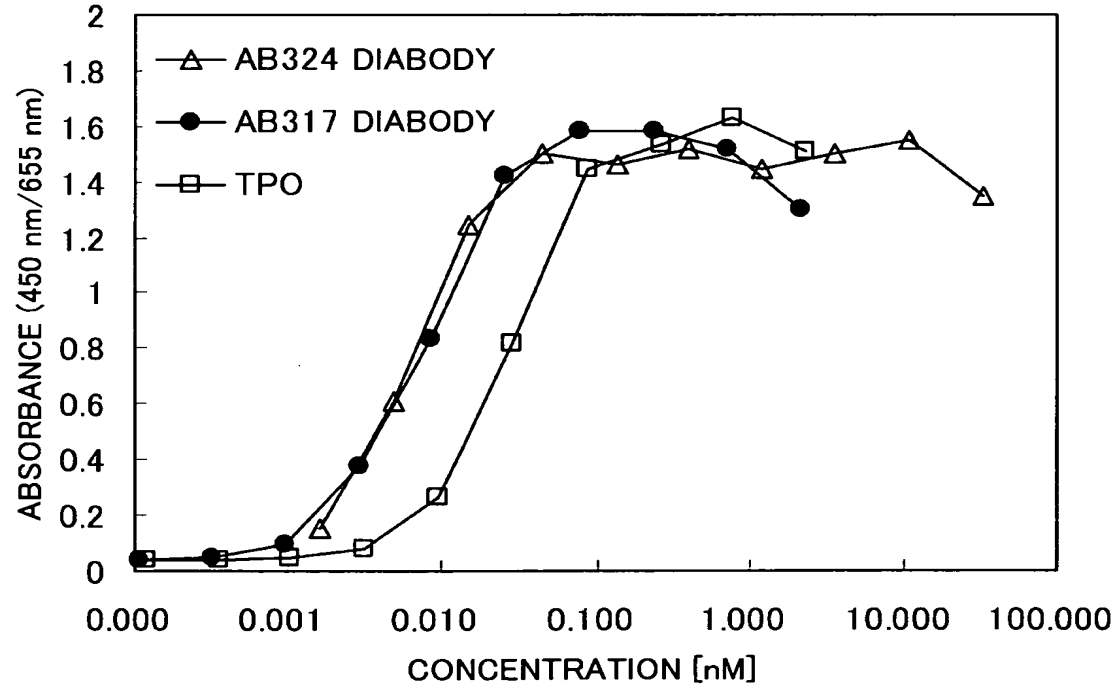
[Fig. 8]


CHO-HUMAN Mpl
CHO-MONKEY Mpl
CHO-MOUSE Mpl
CHO
10⁰
10¹
10²
10³
FITC LOG

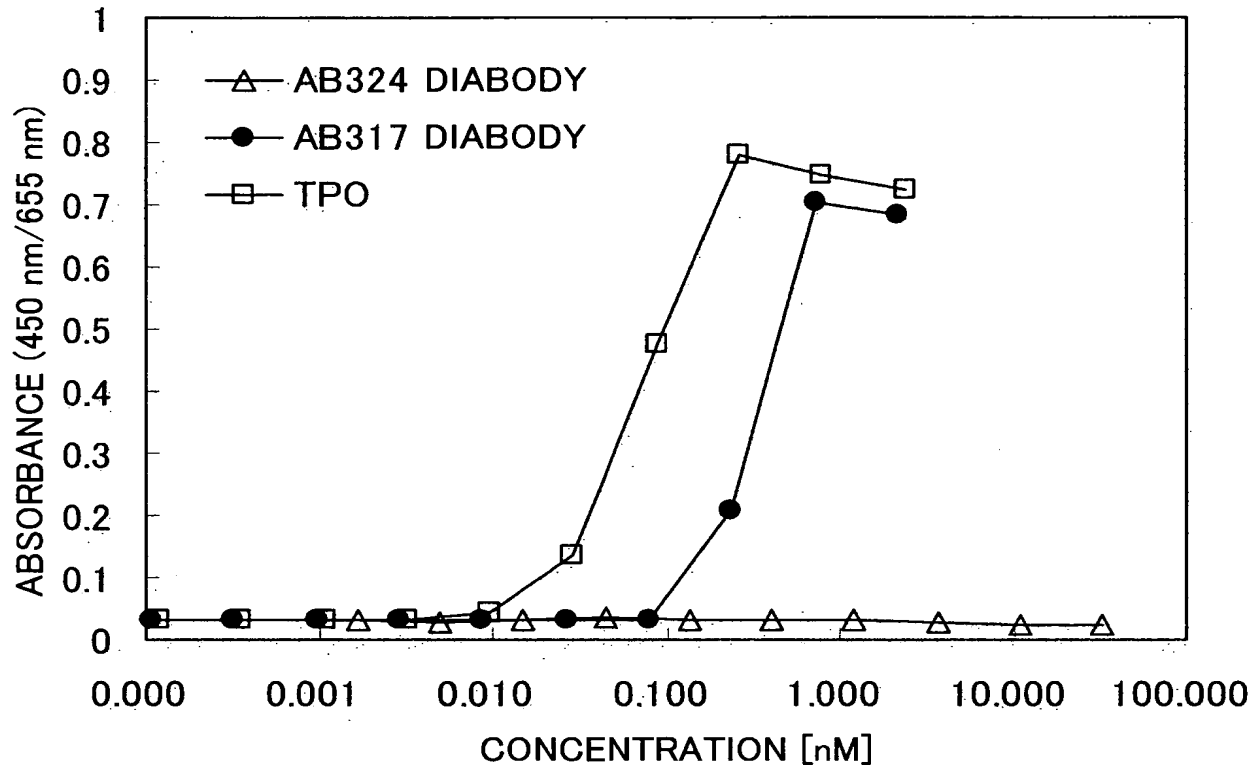
[Fig. 9]


AB324 DIABODY
AB317 DIABODY
TPO
ABSORBANCE (450 nm/655 nm)
CONCENTRATION [nM]
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0.000
0.001
0.010
0.100
1.000
10.000
100.000

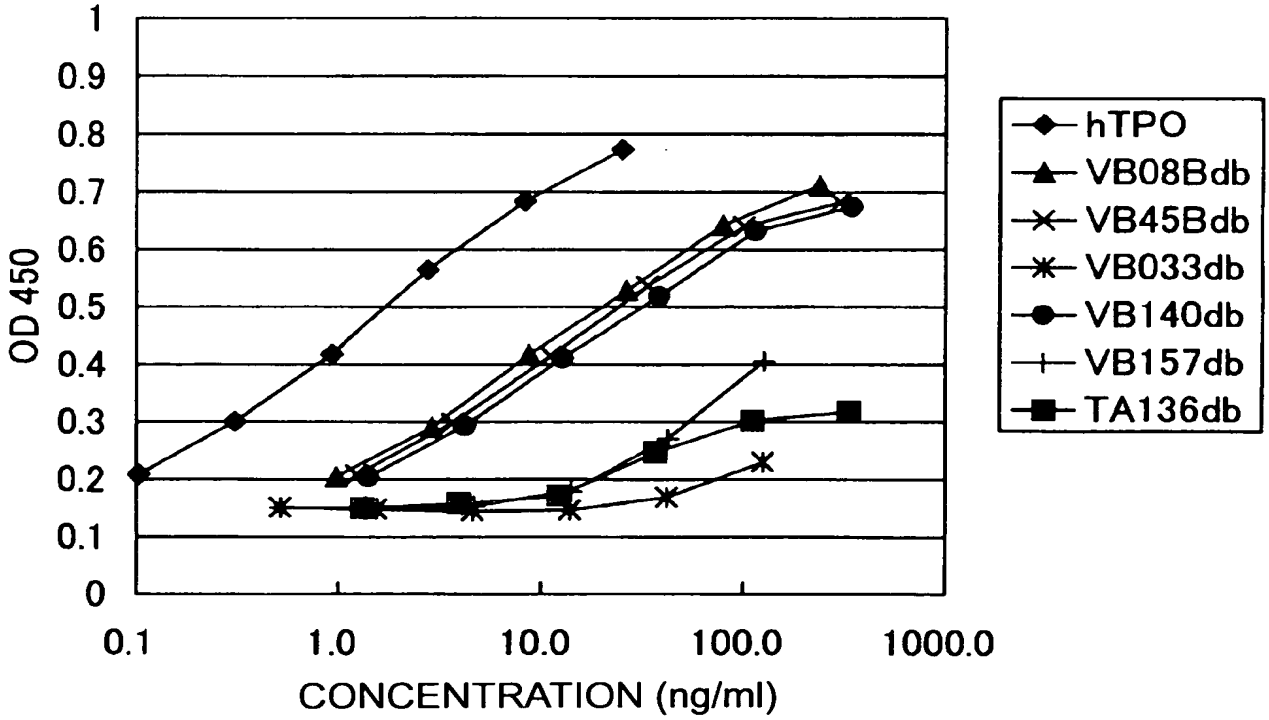
[Fig. 10]


AB324 DIABODY
AB317 DIABODY
TPO
ABSORBANCE (450 nm/655 nm)
CONCENTRATION [nM]
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0.000
0.001
0.010
0.100
1.000
10.000
100.000

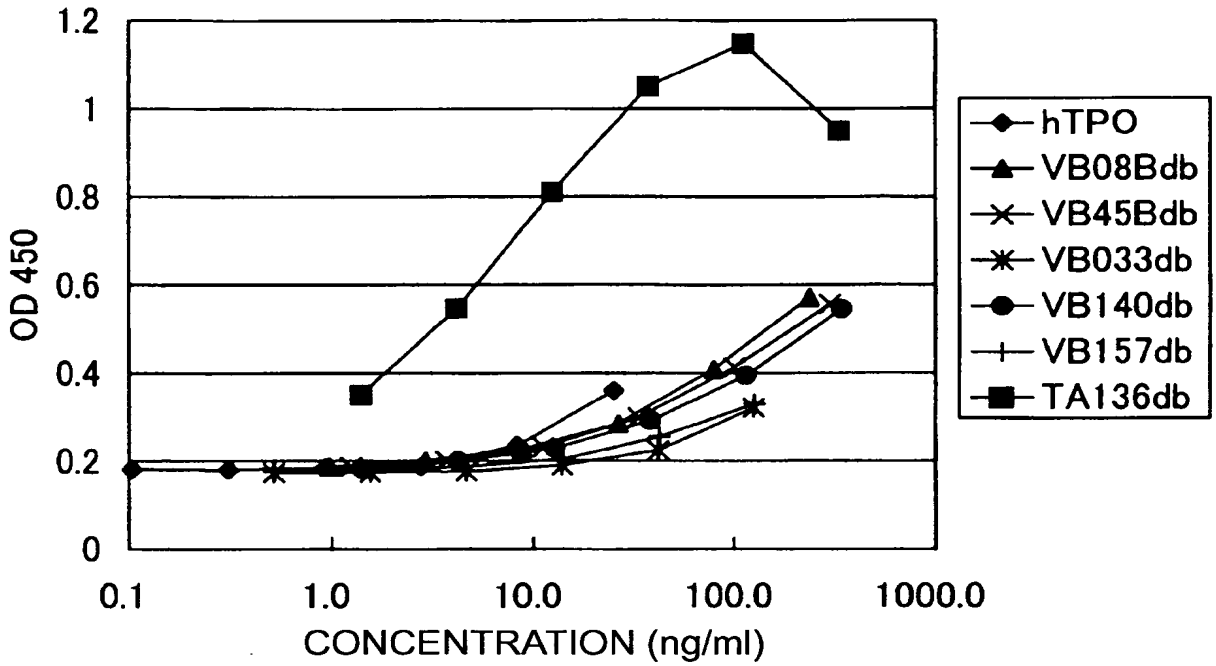
[Fig. 11]


AB324 DIABODY
AB317 DIABODY
TPO
ABSORBANCE (450 nm/655 nm)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.000
0.001
0.010
0.100
1.000
10.000
100.000
CONCENTRATION [nM]

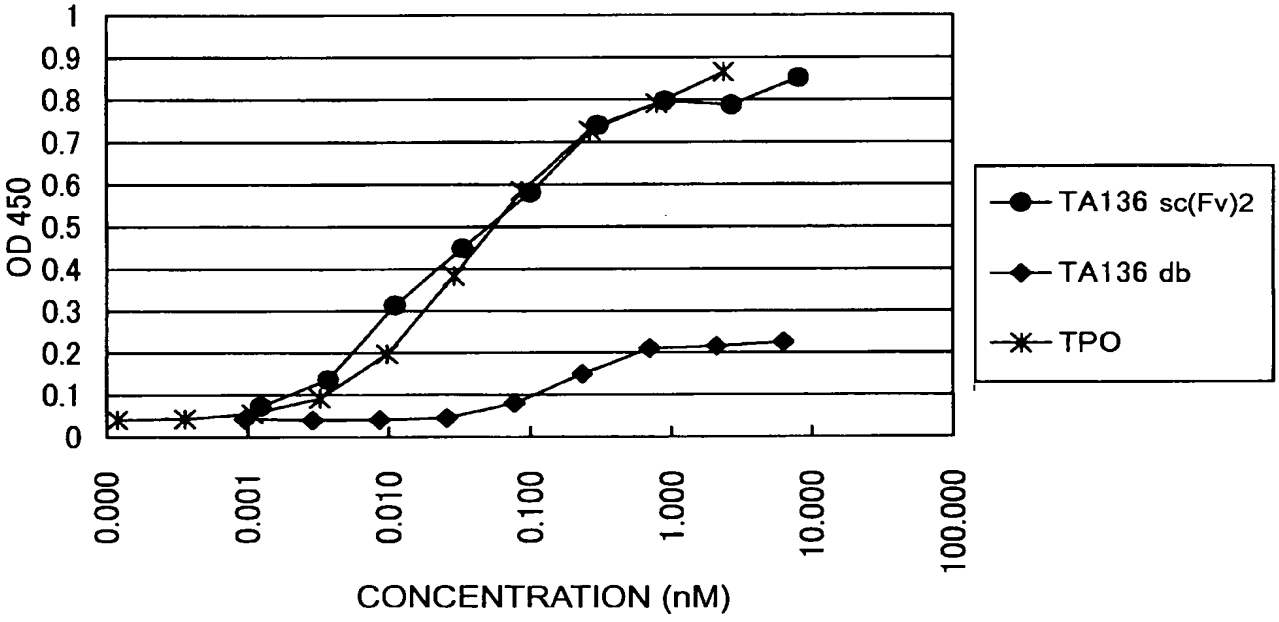
[Fig. 12]


hTPO
VB08Bdb
VB45Bdb
VB033db
VB140db
VB157db
TA136db
OD 450
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.1
1.0
10.0
100.0
1000.0
CONCENTRATION (ng/ml)

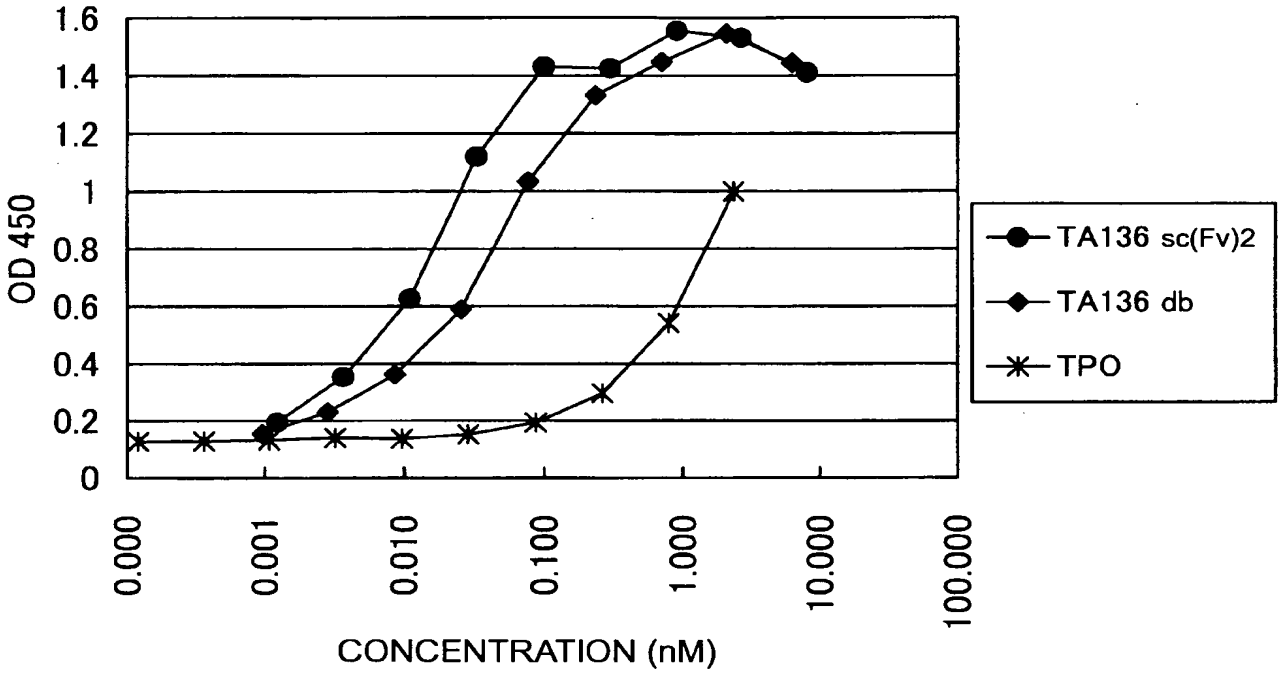
[Fig. 13]


1.2
1
0.8
0.6
0.4
0.2
0
OD 450
0.1
1.0
10.0
100.0
1000.0
CONCENTRATION (ng/ml)
hTPO
VB08Bdb
VB45Bdb
VB033db
VB140db
VB157db
TA136db

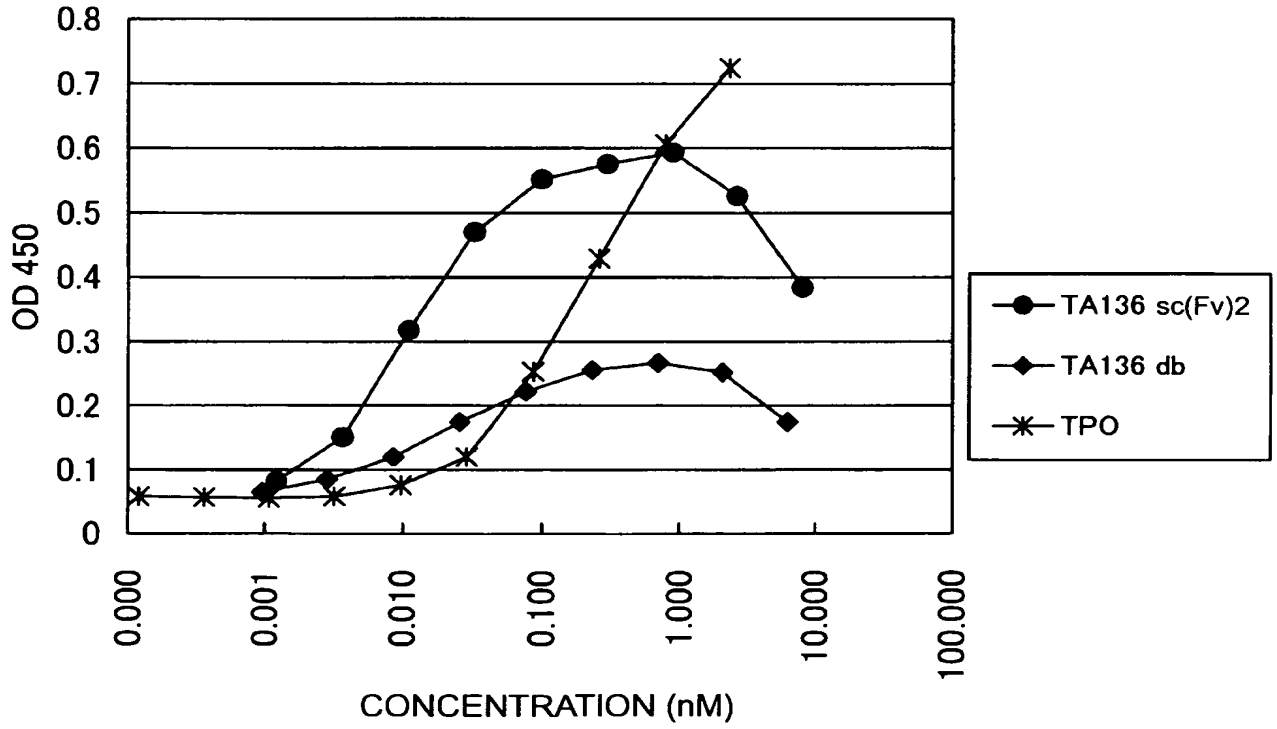
[Fig. 14]


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD 450
0.000
0.001
0.010
0.100
1.000
10.000
100.000
CONCENTRATION (nM)
TA136 sc(Fv)2
TA136 db
TPO

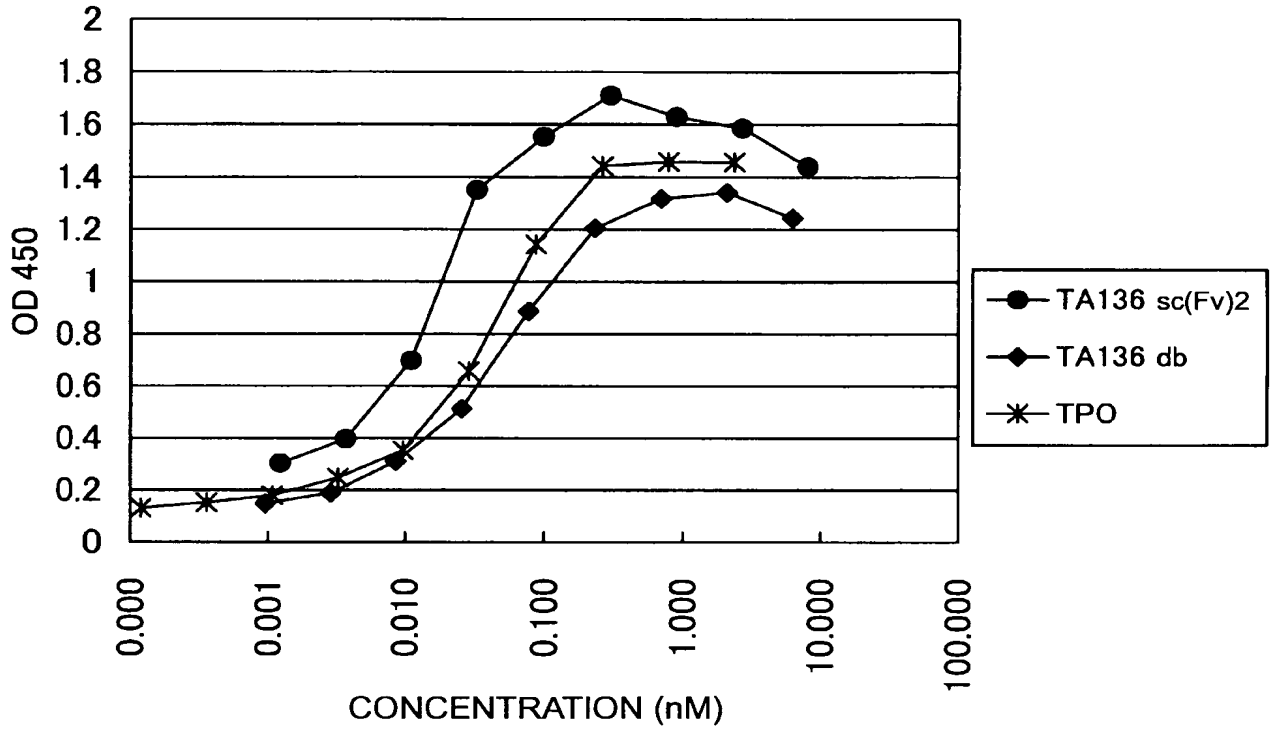
[Fig. 15]


1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD 450
0.000
0.001
0.010
0.100
1.000
10.000
100.000
CONCENTRATION (nM)
TA136 sc(Fv)2
TA136 db
TPO

[Fig. 16]


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD 450
0.000
0.001
0.010
0.100
1.000
10.000
100.000
CONCENTRATION (nM)
TA136 sc(Fv)2
TA136 db
TPO

[Fig. 17]


2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD 450
0.000
0.001
0.010
0.100
1.000
10.000
100.000
CONCENTRATION (nM)
TA136 sc(Fv)2
TA136 db
TPO

[Fig. 18-1]

HUMANIZED HEAVY CHAIN

	FR1	CDR1	FR2	CDR2
hVB22B u2-wz4	QVQLVQSGPEVKKPGASVKVSCKASGYTFT	NSWMN	WVRQRPGKGLEWIG	RIYPGDGETIYNGKFRV
hVB22B q-wz5	QVQLVQSGPEVKKPGASVKVSCKASGYTFT	NSWMN	WVRQRPGKGLEWIG	RIYPGDGETIYNGKFRV
hVB22B p-z	QVQLVQSGPEVKKPGASVKVSCKASGYTFT	NSWMN	WVRQRPGKGLEWMG	RIYPGDGETIYNGKFRV
hVB22B g-e	QVQLVQSGPEVKKPGASVKVSCKASGYTFT	NSWMN	WVRQRPGKGLEWVG	RIYPGDGETIYNGKFRV
hVB22B e	QVQLVQSGPEVKKPGASVKVSCKASGYTFT	NSWMN	WIRQRPGKGLEWIG	RIYPGDGETIYNGKFRV

	FR3	CDR3	FR4	
hVB22B u2-wz4	RVTITADESTSTAYMQLSSLRSEDTAVYYCAR	GYDDYSFAY	WGQGTTVTVSS	(SEQ ID NO: 289)
hVB22B q-wz5	RVTITADESTSTAYMELSSLRSEDTAVYYCAR	GYDDYSFAY	WGQGTTVTVSS	(SEQ ID NO: 295)
hVB22B p-z	RVTITADESTSTAYMELSSLRSEDTAVYYCAR	GYDDYSFAY	WGQGTTVTVSS	(SEQ ID NO: 229)
hVB22B g-e	RVTITADESTSTAYMELSSLRSEDTAVYYCAR	GYDDYSFAY	WGQGTTVTVSS	(SEQ ID NO: 256)
hVB22B e	RVTITADESTSTAYMELSSLRSEDTAVYYCAR	GYDDYSFAY	WGQGTLVTVSS	(SEQ ID NO: 262)

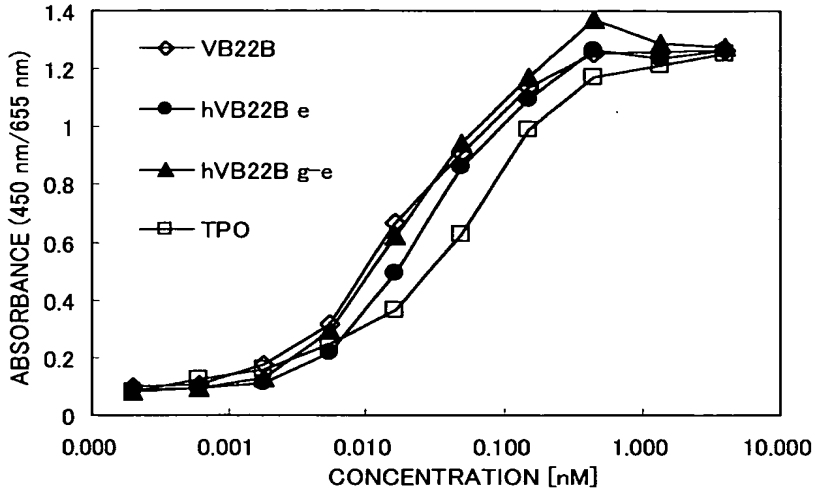
[Fig. 18-2]

HUMANIZED LIGHT CHAIN

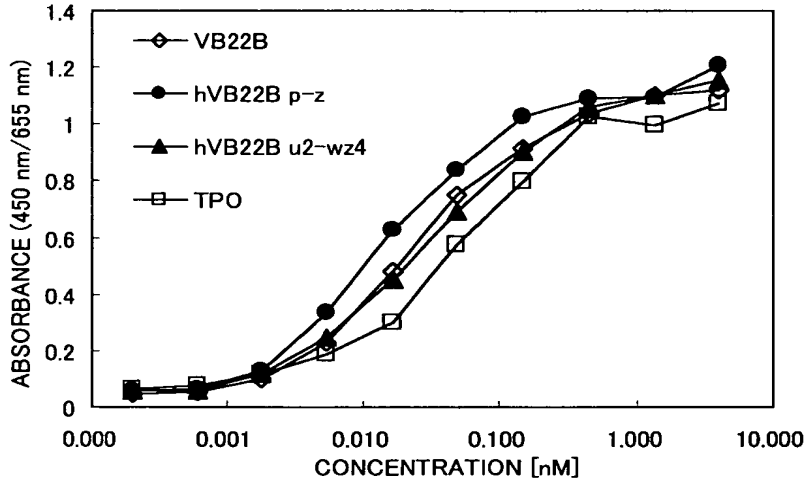
		FR1	CDR1	FR2	CDR2
hVB22B	u2-wz4	DIVMTQSPLSLPVTPGEPASISC	RSSKSLLHSNGNTYLY	WFLQKPGQSPQLLIY	RMSNLAS
hVB22B	q-wz5	DIVMTQSPLSLPVTPGEPASISC	RSSKSLLHSNGNTYLY	WFQQKPGQAPRLLIY	RMSNLAS
hVB22B	p-z	DIVMTQSALSLPVTPGEPASISC	RSSKSLLHSNGNTYLY	WFQQKPGQSPQLLIY	RMSNLAS
hVB22B	g-e	DIVMTQSALSLPVTPGEPASISC	RSSKSLLHSNGNTYLY	WYLQKPGQSPQLLIY	RMSNLAS
hVB22B	e	DIVMTQSALSLPVTPGEPASISC	RSSKSLLHSNGNTYLY	WYLQKPGQSPQLLIY	RMSNLAS

		FR3	CDR3	FR4	
hVB22B	u2-wz4	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQHIEYPFT	FGQGTKLEIK	(SEQ ID NO: 291)
hVB22B	q-wz5	GVPDRFSGSGSGTAFTLKISRVEAEDVGVYYC	MQHIEYPFT	FGQGTKLEIK	(SEQ ID NO: 297)
hVB22B	p-z	GVPDRFSGSGSGTAFTLKISRVEAEDVGVYYC	MQHIEYPFT	FGQGTKLEIK	(SEQ ID NO: 238)
hVB22B	g-e	GVPDRFSGSGSGTAFTLKISRVEAEDVGVYYC	MQHIEYPFT	FGQGTKLEIK	(SEQ ID NO: 258)
hVB22B	e	GVPDRFSGSGSGTAFTLKISRVEAEDVGVYYC	MQHIEYPFT	FGQGTKLEIK	(SEQ ID NO: 258)

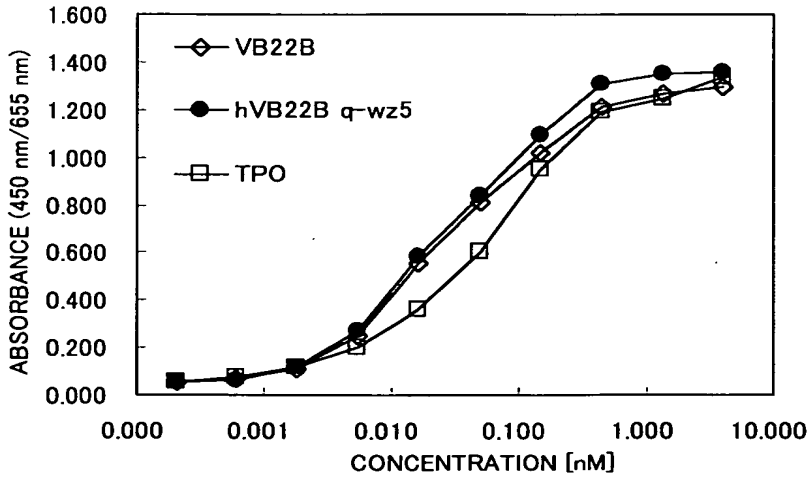
[Fig. 19]



[Fig. 20]



[Fig. 21]



[Document Name] Abstract

[Abstract]

[Problems to be Solved] An objective of the present invention is to provide novel anti-Mpl antibodies having TPO-agonistic activity.

[Means for Solving the Problems] Anti-human Mpl antibodies were isolated and purified, and then anti-human Mpl diabodies and anti-human Mpl $sv(Fv)_2$ were purified using genetic engineering techniques. Furthermore, the present inventors succeeded in humanizing anti-human Mpl $sc(Fv)_2$.

The diabodies and $sc(Fv)_2$ were assayed for TPO-like agonistic activity, and were found to have activities higher than those of anti-human Mpl antibodies, or activities equivalent to or higher than those of naturally-occurring human TPO ligand.

[Selected Drawings] None